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RADIO IODINE: ITS USE AS A TOOL IN THE STUDY OF THYROID PHYSIOLOGY*

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WITH the discovery of artificial radioactivity by Joliot and Curie (1), the building of the cyclotron by Lawrence and his associates (2, 3), and the development of the uranium chain reacting pile (4), the physicists have offered to biologists and physicians valuable tools for the study of intermediary metabolism. Indeed, the discovery of radioactive tracer techniques has been likened in importance (5, 6) to the invention of the microscope. The particular advantage of studies with these techniques lies in their applicability to the investigation of metabolic processes in physiologic equilibrium.

As Marine (7, 8, 9, 10) and his associates demonstrated in 1915 and 1916, the thyroid gland is unique in that it has the ability to collect iodine selectively in relatively large quantities. It is, therefore, not surprising that studies with the radioactive isotopes of iodine have proved readily applicable and illuminating in the study of thyroid physiology.

In 1937, a cooperative investigation for the study of thyroid physiology with radioactive isotopes of iodine was started in the Physics Department of the Massachusetts Institute of Technology and the Thyroid Clinic of the Massachusetts General Hospital. Very soon thereafter a similar project was undertaken at the University of California. Since then investigators in

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institutions throughout the scientific world have used similar techniques for the study of problems related to thyroid physiology. In the laboratory these studies have been done in experimental animals and with in vitro techniques on slices of thyroid tissue. In the clinic radio iodine tracer methods have been applied in studies of normal human beings and of patients suffering from various thyroid maladies.

Five isotopes of radioactive iodine have been described in detail by Livingood and Seaborg (11). They are isotopes produced by bombardment of iodine, tellurium or antimony by neutrons, deuterons or helium ions respectively. The first available radioactive iodine was prepared by the action of slow neutrons on iodine. It has also been prepared by deuteron bombardment of tellurium. Its atomic weight is 128. Its half life of 25 minutes put a limit on the time of observation in the early studies with radioactive iodine. Bombardment of iodine with fast neutrons gives rise to a radioactive element which has a half life of 13 days and has an atomic weight of 126. Chemically, it is identifiable as iodine and therefore, is known as I^{126}. This same isotope has also been prepared by bombarding antimony with helium ions. Another radioactive isotope of iodine arises from the bombardment of antimony with helium ions. Its half life is four days and its atomic weight is 124. The bombardment of metallic tellurium with deuterons gives rise to two radioactive isotopes of iodine. One has an atomic weight of 130 and a 12.5 hour half life. The other has an atomic weight of 131 and a half life of 8 days. The eight day half life of this last named isotope makes it a most convenient preparation for use in biological experimentation. In the literature these various isotopes are identified by their atomic weights or by designating their half life periods.

The Collection of Iodine by the Thyroid and the Effect of the Thyrotropic Hormone.

Hertz and his associates (12, 13, 14, 15) have demonstrated that the normal thyroid collects far greater amounts of administered labelled iodine than do other tissues. They have also observed that thyroids made hyperplastic by the administration of thyrotropic hormone or certain nitriles or by cabbage feeding have a greater affinity for iodine than do normal thyroids. The thyroids of animals previously treated with iodine collect smaller amounts of the administered tracer iodine than do the thyroids of normal animals.

LeBlond and Sue (16, 17, 18) have also demonstrated that thyroid tissue collects selectively more iodine than do other tissues of the body. They have observed that the thyroids of hypophysectomized animals collect much less iodine than do the thyroids of intact animals, but much more than do other tissues of the body. The thyroids of animals previously

treated with thyrotropic hormone collect much more iodine than do the thyroids of normal control animals.

LeBlond (19) has used radioactive iodine in studying the anatomic distribution of iodine in the rat thyroid. The thyroids were removed and sectioned after radioactive iodine had been administered. Radioautographs were made by placing the sectioned thyroids against photographic film. The sections when stained were compared with the shadows produced by emanations from the deposited radioactive iodine. With these studies it has been observed that iodine is deposited primarily in the colloid. In another study with autoradiograms (20), LeBlond has observed a predominance of basophilic follicles in the stimulated thyroids. Yet, he has found that these follicles always contain less of the radioactive iodine than do the acidophilic follicles. He interprets these results as indicating that the turnover of iodine proceeds more rapidly in the basophilic follicles than in the acidophilic follicles.

Ariell and his associates (21), have demonstrated that though the thyroid collects by far the highest percentage of the administered iodine, lung and kidney tissues collect relatively more than do other tissues of the body. They could not demonstrate any radioactivity in the expired air of their animals.

LeBlond and Mann (22) have observed that rats on an iodine deficient diet, or receiving an iodine deficient diet supplemented by soy beans, collect more iodine in their thyroids than do the control animals. The soy bean fed animals have a greater affinity for the administered iodine than do animals on an ordinary iodine deficient diet.

Keating (23) has used radioactive iodine in a collaborative study with the Radioactivity Center of the Massachusetts Institute of Technology and with members of the Thyroid Clinic of the Massachusetts General Hospital. The objective of this study was to determine whether any relationship could be demonstrated between the anatomic changes effected by the injection of thyrotropic hormone and changes in the thyroid's capacity to collect iodine or to release previously stored iodine. The day old male chick was used as the test animal. In each experiment of this study the animals were divided into two sub-groups. One group served for the study of iodine collection, and the other group was used to determine the effect of treatment on the thyroid weight and on the mean acinar cell height done according to the techniques described by Rawson and Starr (24) and Rawson and Salter (25). The animals were injected with 2 gamma of iodine as sodium iodide labelled with radioactive iodine with a half life of eight days (I^{131}) twenty-four hours after the last injection of the thyrotropic hormone. Four hours later the animals were sacrificed and their thyroids removed. The percentage of labelled iodine collected was de-

termined by comparing the radioactivity in an alkaline digest of the thyroids with that in a known amount of the original preparation of radioactive iodine.

In the first experiment 120 chicks were divided into five groups, and the effects of varying doses of thyrotropic hormone were determined. Thyrotropic hormone in doses of $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, and 1 unit was administered by subcutaneous injection for 4 successive days (26). It was observed that with increasing doses of thyroid stimulating hormone both the weights of the thyroid glands and the mean acinar cell heights continue to increase

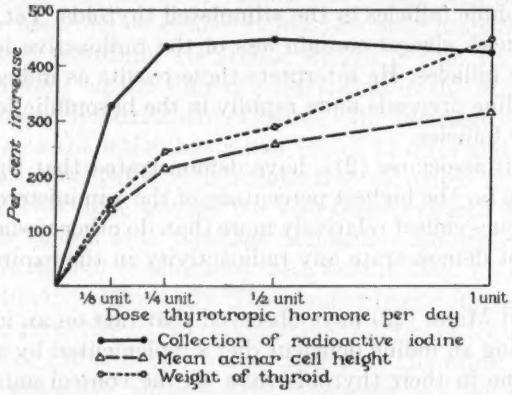


FIG. 1. The effect of varying doses of thyrotropic hormone on the collection of radioactive iodine, mean acinar cell height and on the weight of the thyroids of baby chicks. Each point on the figure represents the average per cent increase over the average observed in untreated controls.

though the latter tend to level off with larger doses. The capacity of the thyroid to collect the labelled iodine apparently reaches a plateau when the daily dose of thyrotropic hormone exceeds $\frac{1}{4}$ of a unit. The average per cent increase over the normal in mean cell height, thyroid weight and iodine collection for each dose group is illustrated in Figure 1.

In the second experiment the effects of repeated doses of thyrotropic hormone were determined on 278 chicks. The treated animals were injected with one unit of thyrotropic hormone daily. Beginning with the first dose animals from the treated and control groups were sacrificed twenty-four hours after each injection. It was observed that the mean acinar cell heights increase rapidly following the initial dose and increase by successively smaller increments following each subsequent dose. The weights of the thyroids increase slowly at first but later increase precipitously. By the fifth day the increase in weight far exceeds the increase in cell height.

Figure 2 illustrates the average increase over the normal in each of these responses as the number of injections with thyrotropic hormone is increased.

It would appear that the anatomic changes produced by administering thyrotropic hormone are, first, an increase in the size of the existing thy-

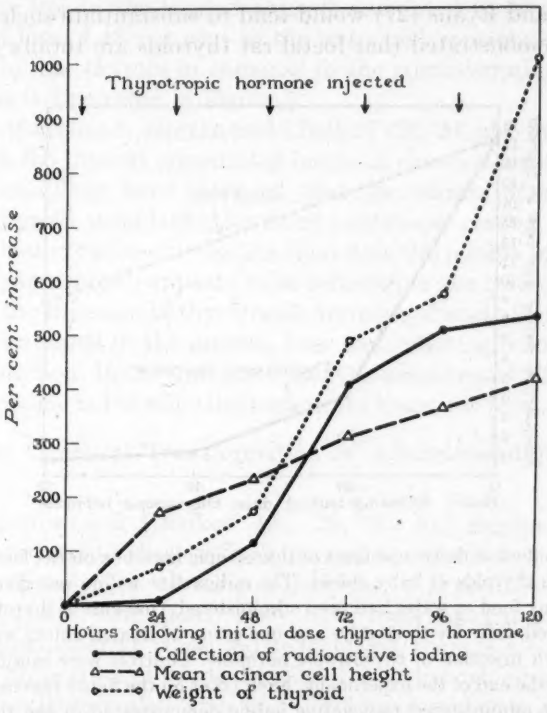


FIG. 2. The effect of daily injections of thyrotropic hormone on the collection of radioactive iodine, mean acinar cell height and weight of the thyroid of baby chicks. One unit of thyrotropic hormone was given daily. One group of chicks was sacrificed 24 hours after each injection. Each point on the figure represents the average per cent increase over the average observed in the untreated controls.

roid cells and, second, a multiplication of cells after the cellular hypertrophy has reached a ceiling. The latter change would account for much of the increase in weight, though some of this weight increase is no doubt due to an increase in vascularity. There is no change in capacity to collect radioactive iodine in the first twenty-four hours. The increase in this capacity is impressive between 48 and 72 hours, after which there is no further increment even though the thyroid mass continues to increase. This same

phenomenon of decelerating increase in the thyroid's avidity for iodine with greater thyroid stimulation has also been observed by LeBlond and Sue. It would seem that rapidly growing and hyperplastic thyroid tissue exhibits a lesser avidity for iodine than does tissue which shows only hypertrophy. This decrease in function may be attributable to the fact that hyperplastic cells are too young to be capable of collecting iodine. The work of Gorbman and Evans (27) would tend to substantiate such an explanation. They demonstrated that foetal rat thyroids are totally incapable of

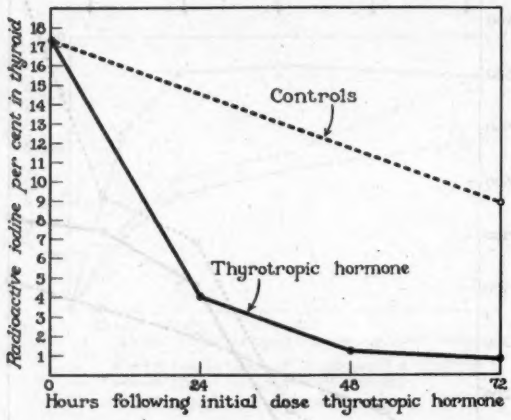


FIG. 3. The effect of daily injections of thyrotropic hormone on the loss of radioactive iodine from the thyroids of baby chicks. The radioactive iodine was given to all of the chicks before any food or water had been administered. One unit of thyrotropic hormone was administered daily for three days and one group of treated chicks was sacrificed 24 hours after each injection of thyrotropic hormone. Controls were sacrificed at the beginning and at the end of the experiment. Each point on the figure represents an average per cent of the administered radioactive iodine demonstrated in the thyroids of each group.

collecting iodine until the nineteenth day. Even then the capacity for collecting iodine is inferior to that observed on the twenty-first day. It is also possible that this apparent decrease in function of the markedly stimulated thyroid is an artifact. The excretion of iodine by the hyperplastic thyroid may be so rapid that the rate of iodine absorption cannot be determined accurately by the methods used in this study. Evidence in support of this explanation may be found in the next experiment (23) in which the effect of the thyrotropic hormone on the release of stored iodine was studied.

Fifty starved chicks, 48 hours old, were given 0.5 micrograms of iodine

labelled with radioactive iodine. The treated animals received one unit daily of thyroid stimulating hormone for three days. One group was sacrificed twenty-four hours after each injection. Controls were sacrificed on the first and third days. The loss of stored iodine was greatly increased by each injection of thyroid stimulating hormone. There was a loss of 76 per cent of the iodine in the first twenty-four hour period. At the end of three days the iodine loss in the treated animals amounted to 96 per cent as compared to a loss of 48 per cent in the untreated controls. The loss of stored iodine by the thyroid in response to the administration of thyrotropic hormone is illustrated in Figure 3.

The studies of Perlman, Morton and Chaikoff (28, 29) also indicate that treatment with the thyroid stimulating hormone causes a rapid release of hormonal iodine. They have observed that the plasma of guinea pigs treated with thyroid stimulating hormone contains a greater percentage of organically bound radioactive iodine than does the plasma of untreated animals. This phenomenon appears to be reflected in the tissues. Twenty-six hours after the injection of thyrotropic hormone almost all of the radioactive iodine contained in the muscle, liver and intestine is found in the thyroxin-like fraction. In the untreated animals more than half of the initially labelled iodine in the same tissues is in the inorganic form.

Studies on the Chemical Transformation of Administered Radioactive Iodine.

Perlman, Morton and Chaikoff (28, 29, 30, 31) employing tracer amounts of radioactive iodine without carrier have executed a series of studies on the distribution of various fractions of thyroid iodine under different experimental conditions. They have reported that as the time interval after administering tracer iodine increases, there is a concomitant increase in the proportion of labelled iodine in the thyroxine-like fraction. These same investigators with Anderson (32) have found that, though the thyroids of hypophysectomized rats collect much less of the tracer iodine than do the thyroids of intact animals, the amount collected is real and measurable. The iodine is readily converted into the diiodotyrosine-like fraction but very little of the tracer is demonstrable in the thyroxine-like fraction.

Mann, LeBlond and Warren (33, 34) have studied the conversion of radioactive inorganic iodine to the diiodotyrosine and thyroxine-like fractions. At 2½, 28 and 48 hour intervals the greatest concentration of the labelled iodine is demonstrable in the diiodotyrosine-like fraction. The longer the time interval the greater is the amount of tracer iodine found in the thyroxine-like fraction.

Morton and associates (35) have studied the formation of thyroxine and

diiodotyrosine by the completely thyroidectomized animal. They have reported that totally thyroidectomized rats are able to convert iodide to diiodotyrosine and thyroxine. (The completeness of the thyroidectomy was confirmed by histologic examination of serial sections from the base of the tongue to the heart and by radioautographic demonstration of the absence of iodine-concentrating tissues in the neck and mediastinum.) As early as 96 hours after its injection, 30 per cent of the radio iodine demonstrable in the liver and in the small intestine is organically bound; 20 per cent is found in the diiodotyrosine-like fraction and 8 per cent in the thyroxine-like fraction. The presence of newly formed radio-diiodotyrosine and radio-thyroxine was established in the thyroidectomized rat by demonstrating a constant radioactivity per unit of crystalline material obtained during several recrystallizations of (1) a mixture of the thyroxine-like fraction of the tissue and inert crystalline thyroxine and (2) a mixture of the diiodotyrosine-like fraction of the tissues and inert crystalline diiodotyrosine.

These observations on the chemical transformation of radioactive iodine are very interesting and may be of fundamental importance. However, it must be remembered that the possibility of an exchange taking place between the natural occurring iodine and the newly administered labelled iodine is real. There is even greater danger of an exchange occurring between the radioactive iodine and added crystalline thyroxine or diiodotyrosine. Miller and co-workers (36) using radioactive iodine as iodide have observed an exchange between the iodide ions and the iodine of diiodotyrosine at a pH of 4 to 5.5. These results do not signify that exchange reactions necessarily occur in vivo among various iodine compounds. However, they do indicate the need for caution in interpreting the results of studies with radioactive iodine on the synthesis and metabolism of the thyroid hormone.

Lein (37) in his studies with radioactive iodine has observed that as inorganic iodine is converted to an organic fraction more total iodine is demonstrable in the gland.

LeBlond, Gross, Peacock, and Evans (38) have observed that rats exposed to cold at the time of maximal stimulation fix 2.7 times as much radioactive iodine in their thyroids as do the control rats. Separation of the iodine fractions of the thyroids at various time intervals indicates that the turnover of thyroxine and the excretion of iodized products proceeds at double the normal rate in animals exposed to cold.

The Effect of Certain Thyroid Inhibitors on the Metabolism of Iodine.

Chaikoff (39, 40) and his associates have utilized radioactive iodine for the study of iodine metabolism with in vitro techniques. They have

incubated thyroid slices in Ringer's solution containing tracer amounts of radioactive iodine and then determined the fractions of inorganic, diiodotyrosine-like and thyroxine-like radioactive iodine in the thyroid tissue. After three hours of incubation as much as 12 per cent of the added iodine in the thyroid slices is in the thyroxine-like fraction and as much as 70 per cent is in the diiodotyrosine-like fraction when slices of rat thyroids are studied. Slices of thyroids from dogs and from sheep convert less of the iodine into the organic fractions. In another study (41) they have reported that cyanide, azide, sulfide and carbon monoxide inhibit the formation of diiodotyrosine and thyroxine. Decreasing the oxygen concentration also inhibits the conversion of inorganic to organic thyroid iodine. They conclude that the formation of diiodotyrosine and thyroxine is linked with aerobic oxidation in which the cytochrome and cytochrome oxidase systems are involved. Cyanide and sulfide inhibit the accumulation of labelled iodine by the thyroid slices, whereas azide has no effect on the collection of iodine. By similar techniques (42) they have demonstrated that various sulfonamides inhibit the formation of diiodotyrosine and thyroxine without affecting the iodine concentrating power of the thyroid slices. In another in vitro study (43) they have observed that the addition of a small excess of iodide to each flask of medium inhibits the conversion of inorganic radioactive iodine to diiodotyrosine-like and thyroxine-like iodine.

With the same in vitro techniques this group of investigators (44) has studied the effect of various goitrogenic substances on the conversion of iodine to diiodotyrosine and thyroxine by slices of sheep thyroids. They have observed that thiourea, thiouracil, allylthiourea, and para amino benzoic acid strongly inhibit the formation of diiodotyrosine and thyroxine but do not prevent the collection of inorganic iodine. Methyl cyanide has no effect on the collection of iodine nor on the conversion of inorganic iodine to the organic fractions. Potassium thiocyanate not only depresses the conversion of inorganic iodine to diiodotyrosine and thyroxine but also prevents the collection of iodine by thyroid slices.

This group of investigators (45) has also studied the influence of thiouracil on the formation of thyroxine and diiodotyrosine by the intact thyroid gland of the rat. The feeding of thiouracil for seven days depresses the uptake of radioactive iodine. The thyroids obtained from rats injected with radio iodine on the last day of thiouracil feeding as well as those removed from rats injected seven days after its feeding had been stopped contained about half the amounts of radio-thyroxine and about one third the amounts of radio-diiodotyrosine found in the normal glands. Recovery, as judged by the iodine concentrating capacity of the thyroid and the amounts of radiothyroxine and radio-diiodotyrosine formed is complete two weeks after the administration of thiouracil has been discontinued.

We have used the radio iodine tracer techniques in studying the mode of action of certain goitrogenic agents on the thyroids of experimental animals and of patients who developed goiters while taking these agents therapeutically.

Since Astwood (46) had shown that the goitrogenic action of potassium thiocyanate can be prevented by iodine and that thiouracil is an iodine resistant goitrogen we (47) undertook to determine whether any difference could be demonstrated in the collection of radioactive iodine by thyroids of animals made goitrous with these two agents. Seventy-five rats were maintained on a Remington borderline iodine deficient diet. The daily intake of iodine in these animals was estimated to be 0.6 gamma. One group of twenty-five rats received thiouracil in the drinking water in a concentration of 0.1 per cent. A second group of twenty-five animals received a 0.25 per cent solution of potassium thiocyanate as drinking water. The remaining 25 animals served as controls. After the experimental animals had received the drugs for a twenty-eight day period, they all received by intraperitoneal injection two gamma of iodine as sodium iodide labelled with radioactive iodine. The animals were sacrificed four hours later. Their thyroids were removed and the radioactivity of an alkaline digest of each thyroid was compared with that of a standard equal to the amount of the injected labelled iodine. The average thyroid weights per 100 Gm. of body weight of the control animals was 10 mg.; of the potassium thiocyanate treated animals 23.0 mg.; and of the thiouracil treated animals 32.0 mg. The average collection of labelled iodine by the thyroids of control animals was 56 per cent; of the potassium thiocyanate treated animals 86 per cent; and of the thiouracil treated animals 10 per cent. The average thyroid weight and per cent collection of iodine by each of these groups is illustrated in Figure 4.

Similar observations have been made on two patients who developed goiters and myxedema while taking these same drugs. The first patient (48) was a hypertensive who developed a large vascular goiter and myxedema after receiving potassium thiocyanate for one year. The second patient was an acromegalic whose growth Drs. F. Albright and E. Reifenstein attempted to decrease by inducing hypothyroidism with thiouracil. Six months after the treatment had started he developed myxedema and a very large vascular goiter. Biopsy specimens were obtained from the goiters of both of these patients. Histologically both were wildly hyperplastic. (See Figures 5 and 6.) Though these goiters were similar in histologic appearance, they differed radically in their capacity to handle radioactive iodine. The thyroid of the patient treated with potassium thiocyanate retained 76 per cent of a tracer dose of radioactive iodine; that of the thiouracil treated patient retained none of it. (See Figure 7.)

It appears that thiouracil blocks the uptake of iodine and thus prevents the formation of an iodinated hormone. The result is a fall in the rate of metabolism and hyperplasia of the thyroid. It has been suggested that the latter is due to increased pituitary thyrotropic activity. Larson, Keating, Peacock and Rawson (49, 50) have demonstrated that thiouracil promptly produces a block to the collection of iodine. They treated 60 chicks with

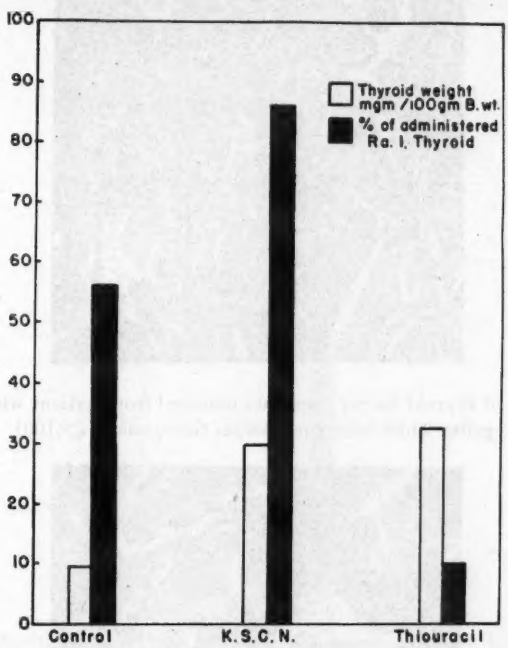


FIG. 4. The collection of radioactive iodine by the thyroids of rats made goitrous by the administration of potassium thiocyanate and thiouracil. The white columns represent the average thyroid weights (mg.100 Gm. body weight). The black columns represent the average per cent collection of administered radioactive iodine by the thyroids.

one unit of thyrotropic hormone daily for four days. On the fifth day the experiment was begun. Forty chicks received 10 mg. of thiouracil by injection at the beginning of the experiment. Groups of 10 animals each received radioactive iodine 1, 6, 12, and 24 hours after the thiouracil had been administered. Ten control animals received radioactive iodine at the beginning of the experiment and were sacrificed four hours later. The remaining 10 control animals received radioactive iodine 24 hours later. The control animals collected 27 per cent of the administered iodine in their thyroids whereas the animals which had received the iodine one hour

FIG. 5. Section of thyroid biopsy specimen removed from patient who developed a goiter while taking potassium thiocyanate. (×100)

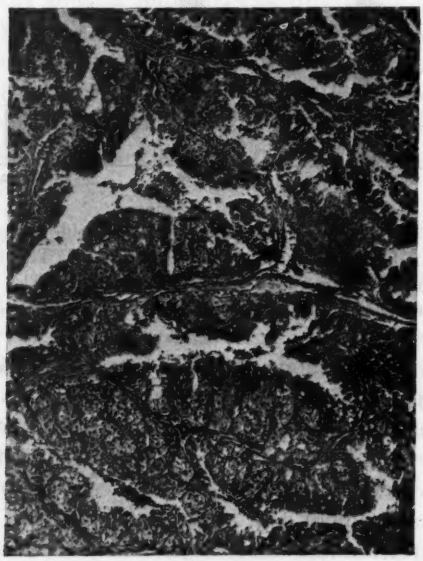


FIG. 6. Section of thyroid biopsy specimen removed from patient who developed a goiter while taking thiouracil. (×100)

after thiouracil treatment collected only 0.7 per cent of the administered iodine. Those animals which had received the radio iodine 12 hours after the thiouracil was administered showed some escape from the thiouracil block to the collection of iodine. Twenty-four hours after the administration of thiouracil, the escape was almost complete. These animals collected

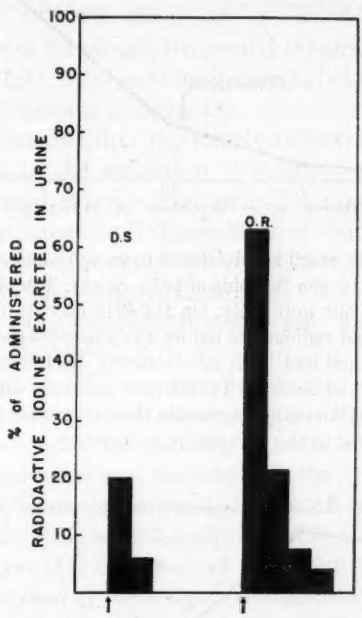


FIG. 7. The excretion in the urine of administered radioactive iodine by patients having struma medicamentosa. The column on left represents the per cent of administered radioactive iodine excreted by a patient who developed a goiter while taking potassium thiocyanate. The column on the right represents the per cent of administered radioactive iodine excreted by a patient who developed a goiter while taking thiouracil

23 per cent of the administered iodine, controls sacrificed at the same time collected 29 per cent of the administered iodine. (Figure 8). The minimal dose of thiouracil necessary to produce this block was found to be 5 mg. (Figure 9).

McGinty and Rawson (51) have determined the minimal dose of various goitrogenic agents necessary to produce a 90 per cent or greater block to the collection of radioactive iodine by the thyroids of rats and chicks. Rats which had been maintained on an iodine deficient diet for a period of weeks simultaneously received varying doses of each drug and then were given a tracer dose of radioactive iodine one hour after administration of

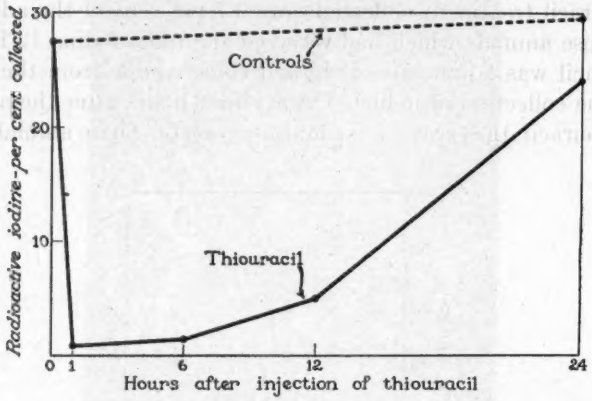


FIG. 8. The effect of thiouracil administered by subcutaneous injection on the collection of radioactive iodine by the thyroids of baby chicks. The chicks received four daily injections of thyrotropin, one unit daily. On the fifth day 10 mg. of thiouracil was administered at zero hour and radioactive iodine was administered at 1, 6, 12 and 24 hour intervals after the thiouracil had been administered. Radioactive iodine was administered to controls, which had received thyrotropic hormone only, at zero hour and 24 hours later. Each point on the figure represents the average per cent of the administered radioactive iodine recovered in the thyroids of each group.

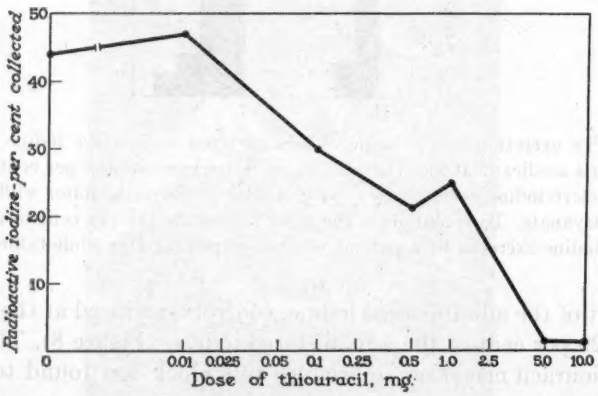


FIG. 9. The effect of varying doses of thiouracil administered subcutaneously on the collection of radioactive iodine administered one hour after the thiouracil had been injected. The chicks were treated with four daily injections of thyrotropic hormone, one unit daily, and the thiouracil was administered on the fifth day. Each point on the figure represents the average per cent of the administered radioactive iodine recovered in the thyroids of each group.

the test agent. Chicks were treated with thyroid stimulating hormone for 4 days and received the test agents in varying amounts on the 5th day. The radioactive iodine was administered one hour later. The per cent block to the collection of iodine was determined by the formula

$$100 = \frac{\%\text{ administered iodine collected by thyroids of test animals}}{\%\text{ administered iodine collected by thyroids of control animals}}$$

The drugs used included thiouracil, 6 n propyl thiouracil, benzyl thiouracil, 5 aminothiazole -2- thiol, 3 (phenyl-aminomethyl) thiazolidine -2- thione, aminothiazole and potassium thiocyanate.

In the rat it was observed that the relative effectiveness of these agents in producing a block to the collection of radioactive iodine descends as follows: benzyl thiouracil, propyl thiouracil, thiouracil, 5 aminothiazole -2- thiol and 3 (phenyl-aminomethyl) thiazolidine -2- thione. In the chick these agents produced a block to the collection of iodine in the following descending order: benzyl thiouracil, propyl thiouracil, thiouracil, 5 aminothiazole -2- thiol, potassium thiocyanate, aminothiazole and 3 (phenyl-aminomethyl) thiazolidine -2- thione.

The same investigators (52) in a parallel chronic study determined the capacity of chick and rat thyroids, made goitrous with the same agents, to collect radioactive iodine. In the goitrous rat the collection of iodine per mg. of tissue in all instances was less than in the controls. The order of capacity to produce a block descended as follows: 3 (phenyl-aminomethyl) thiazolidine -2- thione, thiouracil, 5 aminothiazole -2- thiol, aminothiazole and potassium thiocyanate. In the chick quite different results were obtained. A marked decrease in the collection of iodine per mg. of tissue was observed in animals treated with benzyl thiouracil, propylthiouracil and thiouracil. However, the collection of iodine per milligram of thyroid tissue in chicks made goitrous with aminothiazole, 3 (phenyl-aminomethyl) thiazolidine -2- thione, 5 aminothiazole -2- thiol and potassium thiocyanate was significantly greater than that collected by the controls.

While phenylaminomethyl 2 mercaptothiazoline and 5 aminomercaptothiadiazole in the rat are equal to thiouracil in goitrogenic activity, they are not equal in their capacity to block the collection of radioactive iodine. Therefore, it might be concluded that no necessary correlation exists between the goitrogenic and iodine blocking action of these agents.

The iodine collecting capacity of thyroids made goitrous with various agents appears to be at least partly a function of the time elapsing after the last administration of the agent. This is indicated by the work of Chaikoff and Taurog and of Randall and Rawson. Chaikoff and Taurog (53) have observed that the iodine concentrating capacity of the intact thyroid is depressed in rats treated with potassium thiocyanate provided high con-

centrations of the drug are still present in the circulation. They have also found that 16–24 hours after the last administration of potassium thiocyanate, when potassium thiocyanate can no longer be demonstrated in the plasma, the enlarged gland has a greater than normal capacity for concentrating radioactive iodine. Randall and Rawson (54) have observed a decreased capacity of the thyroids of rats and chicks to concentrate radioactive iodine administered one to six hours following the subcutaneous injection of 10 mg. of potassium thiocyanate. The block was no longer demonstrable after 12 hours.

Radioactive iodine has proved useful in studying the effect of thyroxine on the thyroid. Loeser (55) has postulated that the decreased response of thyroxine treated guinea pigs to injections of thyrotropic hormone is due to a depression of the animal's own pituitary. However, another possibility is that the exogenous thyroid hormone might exert a depressing effect through some direct action of the thyroid cell. With this question in mind, Cortell and Rawson (56) have investigated the effect of thyroxine on the response to thyrotropic hormone in hypophysectomized rats. The response to the thyrotropic hormone was determined by thyroid mean acinar cell height measurements and by the collection of radioactive iodine. Two groups of 19 hypophysectomized rats were studied. One group served as a control; the other group was treated with 20 gamma of thyroxin daily for ten days. Each group of animals was divided into three subgroups, and on the seventh, eighth, ninth and tenth days the animals in each subgroup received 0, 4, and 8 units (26) of thyrotropic hormone respectively. On the 11th day all animals were sacrificed. Their thyroids were examined microscopically under oil immersion. Treatment with thyroxine alone failed to produce any changes in the mean cell height of the hypophysectomized rat thyroids. The mean acinar cell height of animals treated with 4 units of thyrotropic hormone for 4 days was 6.5 micra; of animals treated with the same dose of thyrotropic hormone plus thyroxine, 4.8 micra. The mean acinar cell heights of animals treated with 8 units of thyrotropic hormone was 7.5 micra; of animals treated with this dose of thyrotropic hormone plus thyroxine 6.5 micra.

The capacity to collect radioactive iodine was determined in similarly treated rats. Four groups of hypophysectomized rats were studied. The first group received thyroxine 20 gamma daily for 10 days. The second group was treated with thyroxine 20 gamma daily for 10 days and received 4 units daily of TSH on the seventh, eighth, ninth and tenth days. The third group received TSH only; four units being injected on the seventh, eighth, ninth and tenth days. The fourth group received no treatment. A fifth group served as intact untreated controls. Unoperated untreated control rats were found to collect an average of 6.98 per cent of the adminis-

tered iodine, while hypophysectomized untreated rats fixed only 0.43 per cent, an amount which was not further depressed by the administration of thyroxine. The thyroids of hypophysectomized rats treated with thyrotropic hormone fixed 7.8 per cent of the administered iodine. When the

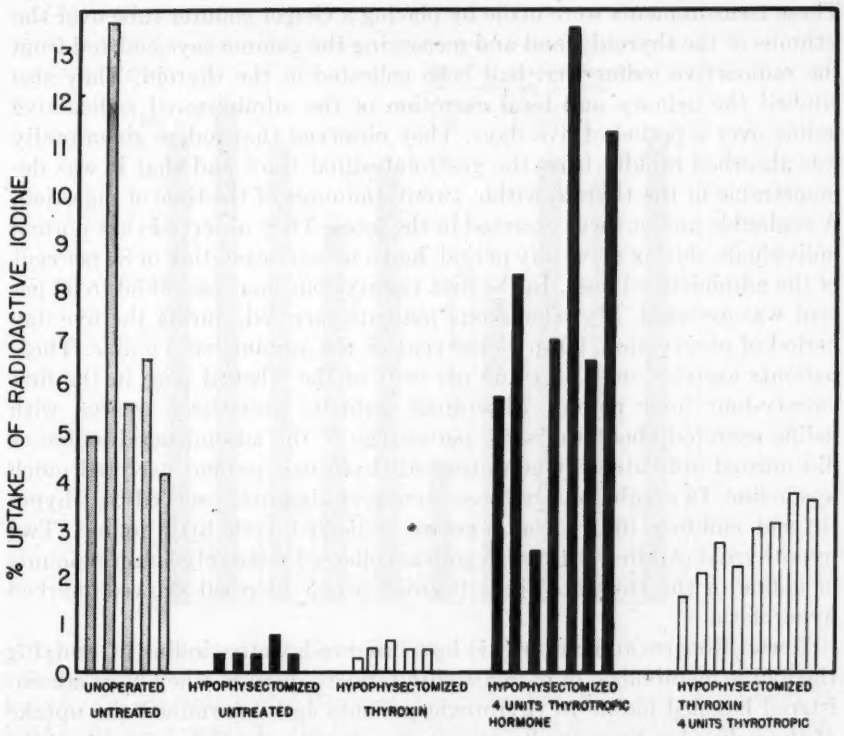


FIG. 10. The effect of thyroxine on the response of the hypophysectomized rats' thyroids to thyrotropic hormone as indicated by the per cent uptake of administered radioactive iodine by the rats' thyroid. Each column represents the per cent uptake of radioactive iodine by a single animal's thyroid gland. Twenty gamma of thyroxine were given daily for 10 days. Thyrotropic hormone was given daily for the last 4 days of the experiment.

thyroxine was administered to hypophysectomized rats along with the thyrotropic hormone the capacity of the thyroid to collect radioactive iodine was depressed to an average of 2.7 per cent (Figure 10). Thus it appears that the administration of thyroid hormone not only depresses the elaboration of the thyrotropic hormone by the pituitary but that it also inhibits the action of the thyrotropic hormone on its end organ.

The Use of Radio Iodine in the Study of Human Thyroid Disease.

Hamilton and Soley (57, 48, 59) were the first investigators to report the use of radioactive isotopes in tracing the course of iodine given to human beings. They compared the uptake of labelled iodine by the thyroids of normal controls and with that of patients with various thyroid diseases. These measurements were made by placing a Geiger counter tube over the isthmus of the thyroid gland and measuring the gamma rays emitted from the radioactive iodine that had been collected in the thyroid. They also studied the urinary and fecal excretion of the administered radioactive iodine over a period of five days. They observed that iodine given orally was absorbed rapidly from the gastrointestinal tract and that it was demonstrable in the thyroid within twenty minutes of the time of ingestion. A negligible amount was excreted in the feces. They observed that normal individuals, during a five day period, had a urinary excretion of 80 per cent of the administered dose. In the first twenty-four hour period 50 to 60 per cent was excreted. Myxedematous patients excreted, during the five day period of observation, 90 to 95 per cent of the administered iodine. These patients excreted only 45 to 50 per cent of the labelled dose in the first twenty-four hour period. Thyrotoxic patients previously treated with iodine excreted about the same percentage of the administered iodine as did normal individuals. One untreated thyrotoxic patient excreted much less iodine. In another study these same investigators observed that hypothyroid children (60) without goiters collected very little iodine. Two hypothyroid children with large goiters collected relatively larger amounts of iodine in the thyroid. These thyroids when biopsied showed marked hyperplasia.

Hertz, Roberts and Salter (61) have used radioactive iodine in studying the iodine metabolism of patients with Graves' disease. They have administered labelled iodine to thyrotoxic patients and determined the uptake of the iodine by direct in situ measurements with the Geiger counter tube and by determination of the urinary excretion of radioactive iodine. The operatively removed thyroids were fractionated, according to the method of Harington, after alkaline hydrolysis and separated into diiodotyrosine and thyroxine-like fractions. They have observed that the untreated hyperplastic thyroid of Graves' disease collects 80 per cent or more of the administered iodine if the dose be small. The initial collection by previously iodinized patients, and by normal controls, is smaller. They have also observed that with increasing time intervals after the administration of labelled iodine there is an increasing amount of radioactive iodine in the thyroxine-like fraction of the gland.

Hertz and Roberts (62) have compared the urinary excretion of a tracer dose of radioactive iodine in patients with classic Graves' disease and in

patients with the hyperophthalmopathic type of Graves' disease. Patients with ordinary thyrotoxicosis excreted less iodine in the urine than did patients with the special ophthalmic complication.

Rawson et al (63) have observed the fate of radioactive iodine administered to 10 patients prepared for thyroidectomy by the administration of

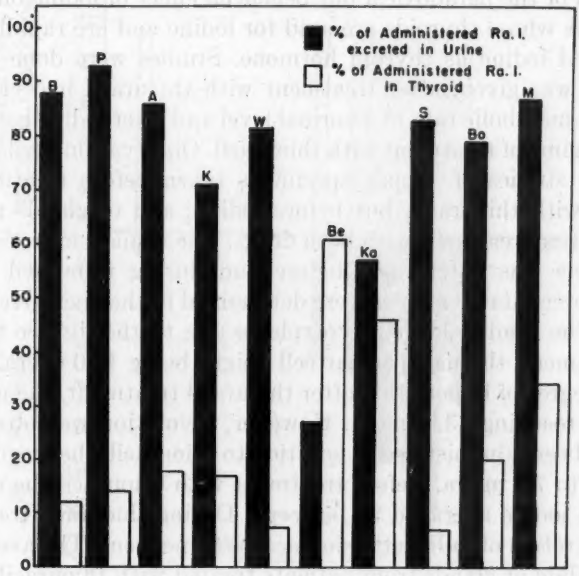


FIG. 11. The excretion in the urine and the collection by the thyroids of radioactive iodine administered to patients with Graves' disease after being prepared for thyroidectomy by the administration of thiouracil. The radioactive iodine was administered 24 to 48 hours before operation. The black columns represent the per cent of the administered radioactive iodine excreted in the urine and the white columns represent the per cent of administered radioactive iodine demonstrated in the operatively removed thyroids.

thiouracil. A tracer dose of radioactive iodine was administered 24 to 48 hours before operation. The radioactivity excreted in the urine and that present in the operatively removed thyroids was determined. In 9 of the 10 cases the greater part of iodine was excreted in the urine and a much smaller amount was demonstrated in the thyroid tissue. These findings are contrasted to observations made on five normal individuals whose urinary excretion averaged 68 per cent of the administered iodine and to those made on four untreated thyrotoxic patients whose urinary excretion averaged 16 per cent of the administered iodine. In Figure 11 is illustrated

the percentage of an administered dose of radio iodine excreted in the urine and the percentage demonstrable in the operatively removed thyroid following treatment with thiouracil.

In another study, Rawson et al. (64, 65) used radioactive iodine tracer technics in patients with Graves' disease undergoing treatment with thiouracil and thiouracil plus iodine. The purpose of this study was to attempt a clarification of the paradoxical but beneficial effect of iodine on hyperthyroid patients whose thyroids are avid for iodine and are rapidly secreting the absorbed iodine as thyroid hormone. Studies were done before any medication was given, after treatment with thiouracil had caused a fall in the basal metabolic rate to a normal level and after iodine had been added to the regime of treatment with thiouracil. Observations included microhistometric studies of biopsy specimens taken before treatment; after treatment with thiouracil, but before iodine; and of glands removed at operation after treatment with both drugs. The urinary excretion of radioactive iodine was determined before and during thiouracil treatment. Total and precipitable iodines were determined in the operatively removed thyroids. The results showed hyperplasia due to the disease before thiouracil treatment, the mean acinar cell height being 12.0 micra. There was a greater degree of hyperplasia after thiouracil treatment, the mean acinar cell height reaching 13.9 micra. However, involution was observed after iodine had been administered in addition to thiouracil, the mean cell height decreasing to 7.2 micra. Before treatment with thiouracil the excretion of radioactive iodine averaged 25 per cent. During thiouracil treatment the average excretion of radioactive iodine was 80 per cent. The average thyroglobulin iodine of glands from patients treated with thiouracil and iodine was 7.0 mg. per 100 Gm. of fresh thyroid tissue. The average thyroglobulin iodine of a comparable series of patients who had been prepared for operation with thiouracil alone was 6.8 mg. per 100 grams of fresh thyroid tissue; of normal human thyroids 29.8 mg. per cent; of a small series of iodine treated patients 36.6 mg. per cent. All of the iodine administered to 1 patient was labelled with radioactive iodine and thus a balance study was made. Before treatment she excreted only 16.3 per cent of a tracer dose. After she had received thiouracil 0.8 Gm. daily for 15 days she excreted 73.5 per cent of an identical dose of radioactive iodine. Following the second biopsy taken from her thyroid, she received a daily dose of 300 mg. of sodium iodide labelled with 100 microcuries of radioactive iodine. The radioactivity demonstrated in the urine excreted during and after the 10 day period of treatment with thiouracil and labelled iodine practically equalled the radioactivity administered. (See Figure 12). The radioactivity demonstrated in the operatively removed thyroid was reported as too little to measure. Although none of the administered iodine was collected

by the thyroid the administration of iodine did cause an involution of the hyperplastic thyroid. (See Figure 13.) It was concluded that iodine exerts two actions on the thyroid gland, an iodinating action and an involuting action in Graves' disease and that these two actions can be separated one from the other by means of thiouracil.

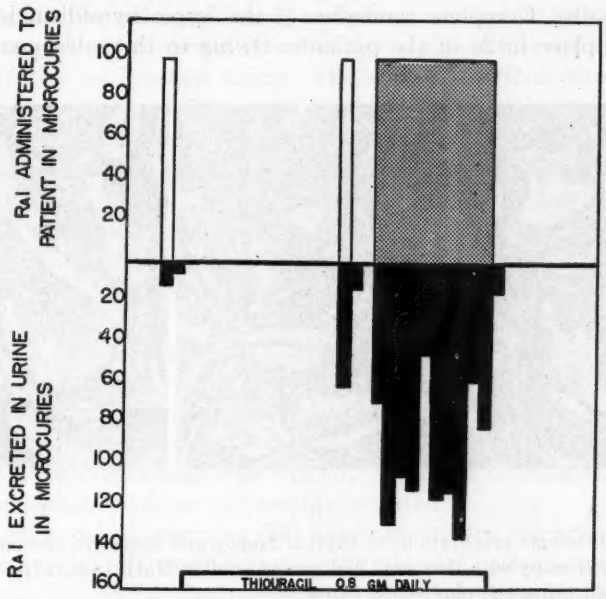
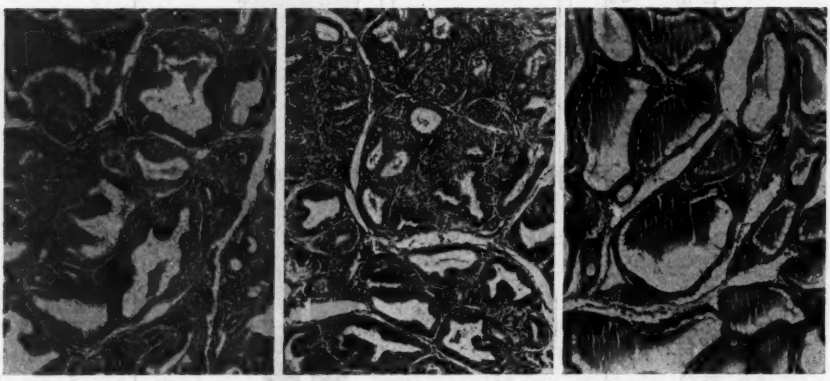


FIG. 12. Radio iodine balance study in patient prepared for thyroidectomy by the administration of thiouracil until the BMR fell to a normal level, then treated with continued thiouracil and added iodine. Intake above the line. Urinary excretion below. With regard to intake, plain columns indicate that tracer iodine was given with carrier of 150 micrograms of sodium iodide. Stippled columns indicate that tracer iodine was given with carrier of 300 mg. daily of sodium iodide. The latter carrier amounted to a therapeutic dose of ordinary iodine.

The Treatment of Hyperthyroidism with Radioactive Iodine.

In 1942 Hamilton and Lawrence (66) reported that they had produced almost complete destruction of the thyroid in dogs and rabbits with radioactive iodine (eight day isotope) without evidence of damage to other tissues of the body. Smaller doses of radioactive iodine were administered to three patients with hyperthyroidism. Two of the patients were in complete clinical remission four and one half months later and the third had required an additional dose of radioactive iodine.

At the same time Hertz and Roberts (67) described the treatment of ten hyperthyroid patients with radioactive iodine. In a supplementary report (68) these authors described the use of radioactive iodine in the treatment of 29 patients with Graves' disease. Doses of 0.7 to 28 millicuries of the 12 hour isotope of radioactive iodine were given, followed by the usual therapeutic dose of saturated solution of potassium iodide for a period of two to four months. Complete remission of the hyperthyroidism is said to have taken place in 20 of the patients. Owing to the subsequent use of



A B C

FIG. 13. Histologic preparations of thyroid biopsy and operative specimens before therapy, after therapy with thiouracil had caused a fall in BMR to euthyroid level and after continued thiouracil plus added iodine.

A. Biopsy before medication. Mean cell height 15.3 ± 0.14 micra.

B. Biopsy after treatment with thiouracil. Mean cell height 17.3 ± 0.17 micra.

C. From operatively removed thyroid after treatment with thiouracil plus iodine. Mean cell height 10.3 ± 0.07 micra. Thyroglobulin iodine 2.3 mg. per 100 GM. of wet tissue.

ordinary iodine, these results cannot command unequivocal acceptance.

Chapman and Evans (69) have also reported on the therapy of Graves' disease with radioactive iodine. They administered radioactive iodine (12 hour isotope) in doses of 14 to 79 millicuries to 22 patients with hyperthyroidism. No additional therapy was employed. Fourteen patients responded well to a single dose of radioactive iodine; three were given two doses and five required three doses. Two patients showed improvement but still had mild hyperthyroidism. Myxedema developed in four patients. Reactions resembling roentgen sickness were observed in six patients who were given large doses of radioactive iodine. Fibrosis of the thyroid was found in biopsies of two patients after radioactive iodine treatment.

The Collection of Radioactive Iodine by Thyroid Tumors.

In view of the beneficial effect of radiation from radioactive iodine in Graves' disease the question naturally arises, "Can this agent be used in treating tumors of the thyroid?" Though the literature on this subject is meagre, activity in this field is considerable.

Hamilton, Soley and Eichorn (70) have studied the anatomic distribution of radioactive iodine in normal and cancerous thyroid tissue by making radioautographs of thyroids removed from patients two days after the administration of labelled iodine. The stained sections were compared with shadows produced by emanations from the deposited radioactive iodine. They observed that practically none of the iodine is deposited in cancerous tissue. The iodine collected by the normal tissue is deposited in the colloid.

Frantz, Ball, Keston and Palmer (71, 72) have administered tracer doses of radio iodine to three patients having thyroid tumors with bony metastases. The uptake of iodine was determined by means of the Geiger counter. The colloid-containing bony metastases of two of the tumors (one, a Hürthle cell tumor; the other, a well differentiated adenoma malignum) failed to take up radio iodine. The third tumor was a carcinoma of the adenoma malignum type with widespread bony and visceral metastases. Only one of the bony metastases showed an appreciable uptake of radioiodine. At autopsy this metastasis was well differentiated histologically, whereas the other metastatic tissue was undifferentiated.

Marinelli and his associates (73) have determined the uptake of radioactive iodine by 19 carcinomas of the thyroid, employing the radioautographic technique. Ten of the tumors took up a detectable quantity of radioactive iodine. Five of these were examples of the "benign metastasizing struma," five had the structure of follicular adenocarcinoma, and one was a highly malignant-appearing solid alveolar carcinoma. In those cases yielding negative results, there were three examples of Hürthle cell carcinoma and the remainder were either solid alveolar or more anaplastic carcinomas. Inasmuch as the tumors studied represent a selected group of cases, the authors emphasize the fact that the figures quoted are somewhat misleading. If the frequency distribution of the various types of thyroid carcinoma be taken into consideration, roughly 15 per cent may be expected to accumulate radioactive iodine in some degree. In no case of "benign metastasizing goiter" was a negative result obtained. Nearly the same statement can be made from this study in regard to orderly follicular carcinoma, but the avidity of the cells of this type of tumor for iodine does not approach that of metastasizing struma. There was a striking lack of uniformity in the pick-up pattern of various thyroid carcinomas that are functional.

McArthur and Cope (74) administered radioactive iodine preoperatively to 18 patients with discrete nodules of the thyroid, and compared the radioactivity of digests of the tumor tissue with digests of the adjacent uninvolved tissue. Of the 18 tumors, 12 were benign. They included four involutional nodules, four struma nodosa micro et macrofolliculare, two fetal adenomas, one papillary adenocystoma and one Hürthle cell adenoma. The iodine collected by the less differentiated tumors, such as the fetal adenomas and the Hürthle cell adenoma, was one fiftieth to one hundredth of that collected by the uninvolved portion of the gland. Four of the differentiated tumors collected more iodine than the uninvolved tissue, one twenty times as much. Two other differentiated tumors absorbed all of the iodine, the uninvolved tissue none; the patients had mild hyperthyroidism. The six malignant tumors included papillary adenocarcinoma and alveolar adenocarcinoma. These collected from none to one fourth of the radioactive iodine taken up by the adjacent uninvolved tissue. In three instances there was a detectable collection of iodine by the tumor metastases.

LeBlond and his collaborators (75) compared nodules and surrounding thyroid tissue in regard to iodine content and to the entry of radioactive iodine into the various iodine fractions. Thyroid adenomas were functionally less active than the surrounding uninvolved tissue, as shown by a lower iodine content, a smaller fixation of radio iodine and a slower turnover of the iodine. The difference was greatest in one case of fetal adenoma, but was also demonstrable in varying degrees in four colloid nodules.

Leiter *et al.* (76, 77) have employed radioactive iodine as an indicator of the physiological activity of the metastatic lesions in two patients with adenocarcinoma of the thyroid and hyperthyroidism. Thyroxine iodine was demonstrated in the metastases of both patients. In one patient, the absence of functional thyroid tissue in the neck, shown by the lack of uptake of radioactive iodine, indicated that the metastases were responsible for the hyperthyroidism. Iodinization failed to check the increasing hyperthyroidism of one patient and only slightly decreased the toxicity of the other. Thiouracil induced a clinical remission, decline in the basal metabolic rate and blood protein-bound iodine, and an increase in plasma cholesterol and body weight. There was a prompt recurrence of hyperthyroidism in both patients upon withdrawal of thiouracil. When deep roentgen therapy proved ineffective, several massive doses of radio-iodine were administered to one of these patients. A striking improvement in the clinical picture resulted: the basal metabolic rate dropped to below normal, the patient gained weight and the growth of the metastases appeared to be arrested.

A subsequent report (78) describes in greater detail the radioactive iodine therapy administered to one of these patients, now followed for

three years. When only 30 to 40 per cent of a tracer dose was shown to be excreted in the urine over a 72 to 96 hour period, treatment with radioactive iodine appeared practicable. Radioautographs of a biopsy of one of the tumor metastases after the administration of a large tracer dose of radioactive iodine likewise showed heavy radio iodine concentration in the tumor tissue. A total dosage of 110.8 mc. of 12 hour iodine and 158 mc. of 8 day iodine has been administered to date. Though the patient exhibits marked clinical improvement and arrest in growth of the metastases, tracer studies still show localization of radio iodine in the metastatic lesions. Further therapy is being planned.

From studies thus far reported it seems that therapy with radioactive iodine in thyroid cancer will be possible only in exceptional cases unless means can be found to increase the avidity of thyroid tumors for iodine.

SUMMARY

Radioactive iodine has proved itself a valuable tool for the study of thyroid physiology. Certain principles of thyroid function which were demonstrated with the classic biochemical techniques have been reenforced and amplified. Progress toward the solution of the steps involved in the synthesis of the natural thyroid hormone has been facilitated. The mode of action of certain goitrogenic thyroid-inhibiting agents has been clarified.

At the clinical level, knowledge of iodine metabolism in Graves' disease has been advanced. Some correlation between the structure and function of benign and malignant tumors of the thyroid has been indicated. In adequate dosage radioactive iodine has proved to be a therapeutic agent of value in certain selected cases of hyperthyroidism and in exceptional instances of thyroid malignancy.

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THE EFFECT OF TRAUMA AND DISEASE ON THE URINARY 17-KETOSTEROID EXCRETION IN MAN[1]

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IN 1941, in reporting a study of the normal variation in the urinary 17-ketosteroid excretion, Fraser, Forbes, Albright, Sulkowitch and Reifenstein (14) remarked upon a decrease in some of the subjects following various kinds of injury or stress. In a preliminary report a year later, Forbes (13) gave a few more examples of this phenomenon and speculated upon its possible relationship to the "Adaptation Syndrome" described by Selye (26). Since that time we have studied the problem further, and have accumulated a substantial body of data which it is the purpose of this article to present.

The method of total neutral 17-ketosteroid assay employed in these studies has already been described (Fraser, *et al.* (14)). The sources of the steroids measured are the testis and the adrenal cortex in males, and the adrenal cortex alone in females. Hence, the test provides a chemical measurement which is an index, especially in females, of at least one aspect of adrenal function.

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During a clinical survey involving some 4,000 determinations of the urinary 17-ketosteroid excretion of normal and abnormal people, we observed that the output of chronically ill or severely undernourished patients is remarkably low. We found also that members of the laboratory staff, whose basal excretion was well known and very constant, sometimes put out as little as 30 per cent of their normal amount following an acute infection. Figure 1 shows the behavior of the urinary 17-ketosteroid level during an episode of subacute appendicitis which developed in one worker whose urine we happened to be assaying daily. The lowered steroid output appeared before the symptoms, and constituted the first evidence of ill-

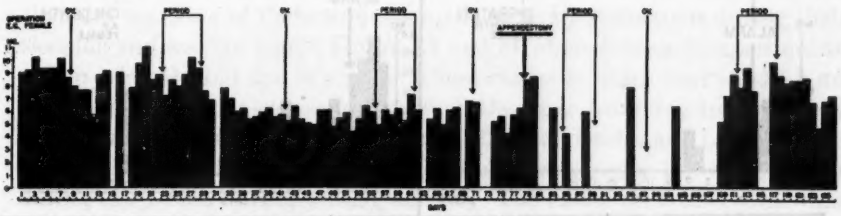

FIG. 1. The urinary 17-ketosteroid excretion of a normal woman during an episode of subacute appendicitis.

ness. Operation caused an abrupt peak in the output followed by a drop to an even lower level than before and a gradual return to the baseline during convalescence. Figure 2A, 2B, and 2C show the findings in three normal individuals undergoing operation, childbirth, and an acute infection, respectively. The line marked "Alarm" represents the day of the injury or of the onset of the disease. These last three charts do not show the initial peak seen in Figure 1 possibly because assays were not made every 24 hours, but all of them are similar in that the 17-ketosteroid excretion is eventually markedly depressed.

These early observations led us to believe that the 17-ketosteroid excretion might be regularly affected by any sort of damage to the body and might be employed to obtain valuable information about the function of the adrenal cortex following damage. With this in mind, we reviewed the data already in our files and began to add to them at every opportunity. We now have studies of the urinary 17-ketosteroid excretion of more than one hundred patients who have been followed through some sort of medical or surgical injury.

I. The 17-Ketosteroid Excretion of Normal Subjects.

We have made assays on 73 normal young men and 65 normal women with regular menstrual cycles. The excretion of normal men fell between

the extremes of 6.7 and 27.2 mg. of steroid per 24 hours; and those of the women between 3.8 and 16.9 mg. per 24 hours. The average for men was 12.5 and for women 8.2 mg. per 24 hours. These values correspond fairly closely with those for normal individuals reported by other investigators (Chou and Wang (9); Callow, Callow, Emmens, and Stroud (8); Fried-

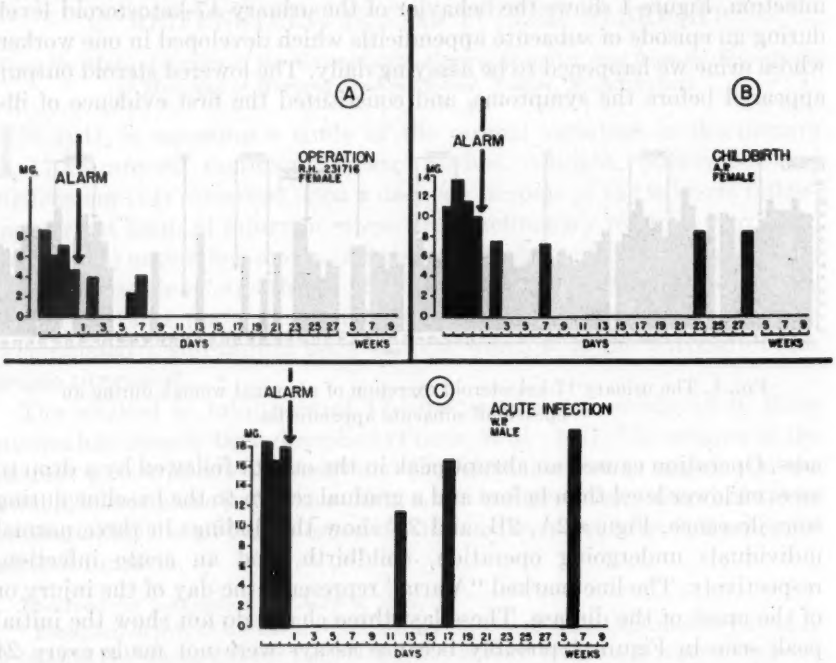

FIG. 2. The urinary 17-ketosteroids of three normal individuals undergoing operation (A), childbirth (B), and acute infection (C).

good and Whidden (17); Baumann and Metzger (5); Talbot, Butler, Berman, Rodriguez, and MacLachlan (30); Werner (32); and Engstrom and Mason (12)). We do not have extensive data on the relationship of the 17-ketosteroid excretion to age; what little we do have show that the level in adults decreases somewhat with increasing age but not markedly until well after the climacteric (Fraser, *et al.* (14)). We could not establish any significant relationship to height, weight, complexion or creatinine excretion.

The amount of scatter between apparently similar individuals is very great and rather baffling. For instance, within a group of 30 student nurses who had all passed medical examinations and in whom the age, diet, and

activity were all quite uniform 47 assays varied from 3.8 to 16.9 mg. per 24 hours (average 7.5 mg.) with a standard deviation from the mean of 3.4 mg. Although the extremes may represent inaccurate collections or minor illnesses, the wide variation indicates that a single 17-ketosteroid determination must be very high or very low to have any diagnostic value.

The results of repeated assays on the same individual are much more consistent. In 17 of the nurses just mentioned, on whom the assay was repeated, the standard deviation between the first and the second determinations was 3.0 mg. in contrast to the 3.7 mg. above. Similarly, in seven normal laboratory workers, who brought in daily collections for one month, the standard deviation from the mean of each individual series was less than 2.5 mg. Five of these subjects made separate collections during their sleeping and waking hours. Figure 3A and 3B show two such experiments, one in a female and one in a male. These charts indicate that the 24-hour excretion in any one case remains much the same from day to day. They bring out another fact, which Pincus (22) and Bachman, Leekley, and Winter (4) have also observed, namely, that the excretion is lower per hour during sleep than during waking activity. The excretion is also somewhat more constant during sleep. The standard deviation from the mean for the day-collections in the two people illustrated in Figure 3 (see Table 1) is 1.9 mg. for the female (whose average excretion was 8.6 mg. per 24 hours for the day), and 2.3 mg. for the male (whose average excretion was 13.2 mg. per 24 hours for the day). During sleep, the female averaged 7.5 mg. per 24 hours with a standard deviation of 1.2 mg.; and the male averaged 10.3 mg. per 24 hours with a standard deviation of 2.3 mg. This difference obtains in spite of the fact that the sleeping hours were about half as many as the waking hours, so that the results are multiplied by approximately twice as much in calculating the 24-hour level. If two sleeping collections are pooled to make one specimen of approximately 16 hours of collection, the result is very constant. The standard deviations (see Figures 3A and 3B) then become 0.8 and 1.9 mg., respectively. The same fact appears on analyzing the other charts. The values in females suggest a slight drop in excretion at the time of ovulation, but we have not made any special allowance for this. Table 1 summarizes the results on all seven subjects, and leads to the conclusion that the sleeping excretion is 12 to 22 per cent lower than the waking, and almost twice as constant. Table 2, previously reported elsewhere by Reifenstein (23) contains confirmatory data analyzed in terms of groups rather than individuals.

Wooster (34) made a statistical comparison of the sleeping excretion calculated on a 24-hour basis with the total 24-hour excretion and found that the sleeping excretion is irregularly lower and more variable than the 24-hour excretion. Werner (33) found that the day to day fluctuations are

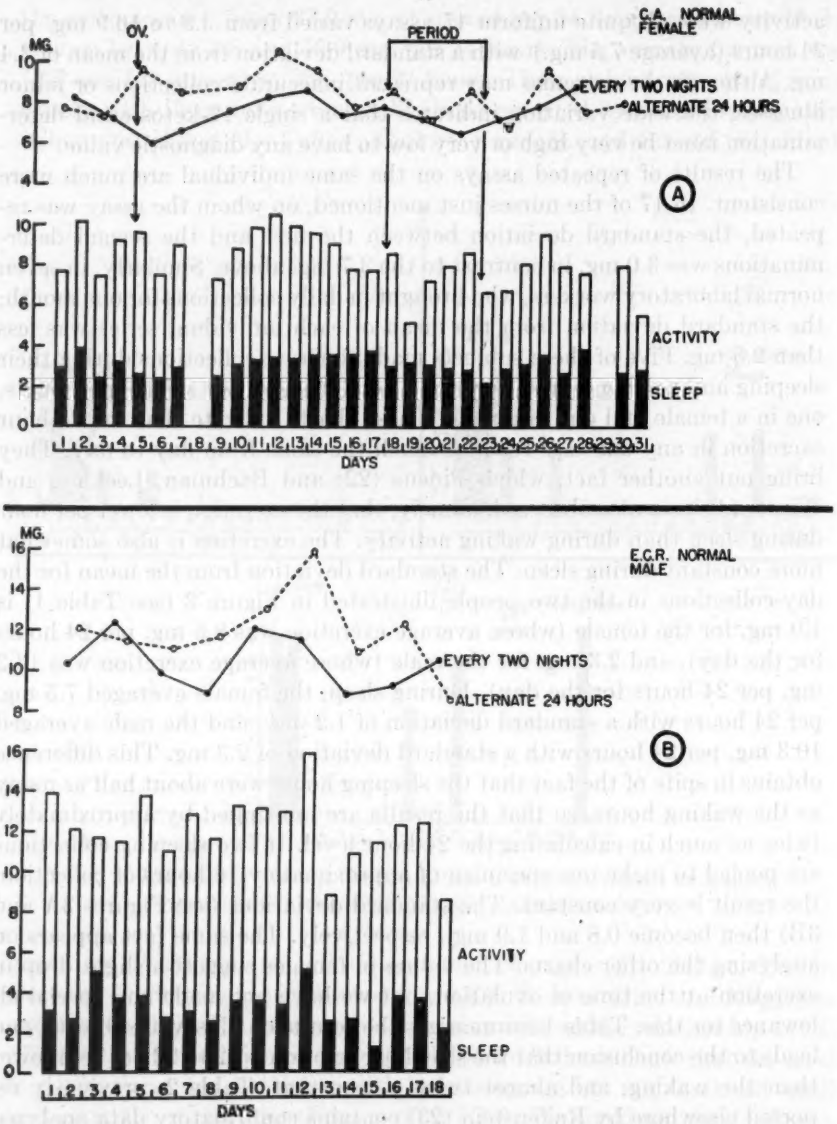

FIG. 3. The urinary 17-ketosteroid excretion per hour during the day and during sleep by a normal woman (A) and by a normal man (B).

TABLE 1. THE WAKING AND THE SLEEPING URINARY EXCRETION OF 17-KETOSTEROIDS IN SEVEN NORMAL SUBJECTS STUDIED DAILY FOR ONE MONTH

Subject	Sex	Average Day Level mg./24 hr.	Average Night Level mg./24 hr.	Difference of Day from Night %	Standard Deviation		
					Day	Night	Two Nights
C.A.	f	8.6	7.5	12.8	1.9	1.2	0.8
E.C.R.	m	13.2	10.3	22.0	2.3	2.3	1.9
E.T.R.	f	8.9	7.7	13.5	1.0	1.3	1.3
E.C.D.	f	8.7	6.8	21.8	1.7	1.1	0.9

Subject	Sex	Average of Daily 16-Hour Collections mg./24 hr.	Standard Deviation
E.C.D.	f	8.1	1.3
R.M.S.	f	7.4	1.3
A.P.F.	f	10.6	1.1

TABLE 2. VARIATION IN THE AVERAGES OF THE WAKING AND SLEEPING URINARY EXCRETION OF 17-KETOSTEROIDS OF A SERIES OF REPEATEDLY ASSAYED INDIVIDUALS

Type of Collection	Subjects			Assays		
	Number	Sex	Age Range yr.	Number of Determinations	Range of Individual Averages mg./24 hr.	Average of Individual Averages mg./24 hr.
16 hours from 4 P.M. to 8 A.M.	10	f	23–37	149	5.7 to 10.7	8.2
	10	m	24–42	46	11.9 to 15.4	13.6
±8 hours while asleep	6	f	24–37	121	5.1 to 10.1	7.5
	5	m	29–42	36	8.2 to 15.7	11.9
±8 hours while awake	3	f	24–31	11	6.5 to 11.5	9.0
	4	m	32–42	23	10.2 to 20.2	15.2
±16 hours while awake	3	f	24–37	62	5.2 to 12.2	8.7
	1	m	32	17	11.4 to 14.4	12.9
Total of all types	12	f	23–37	343	5.6 to 11.1	8.4
	11	m	24–42	124	11.4 to 16.4	13.9

still observed even when the extracts are purified with Girard's reagent T so that only the ketonic fraction is measured.

In conclusion there appears to be a basal ketosteroid excretion which is more or less a constant characteristic of a given individual as is the resting blood pressure. However, the deviations from this basal level brought about by daily activities are not very great. Therefore, although in most of our recent cases two "overnight specimens" have been used instead of one 24-hour collection, any major change in 17-ketosteroid level appears in the 24-hour collection as well, and we have used the latter where it was more convenient to do so.

II. The 17-Ketosteroid Excretion in Patients with Chronic Debility.

The order of magnitude of the changes in ketosteroid excretion produced in an individual by disease is much greater than the usual daily variation. Moreover, chronically ill or severely undernourished people usually have an excretion that falls well below the normal level in spite of the wide range which must be accepted as normal. Table 3 shows the average daily urinary excretion of 196 medical patients taken without selection from the files and grouped by diagnoses. Although we did not ourselves examine each of these patients and supervise the collections for accuracy, as we did in the individual case studies which follow (vide infra), we believe that the deviations from normal are significant and are not explained by faulty collections. The average excretion of all the males in this group is 6.2 mg. per 24 hours as contrasted with the average normal value of 12.5 mg., and of the females 4.4 mg. as contrasted with 8.2 mg. Even when the level of a given patient is not below the minimum found in normals, it may prove to be abnormally low for that individual when further determinations are made after recovery. Table 3 includes some debilitated patients who later improved and were tested again when they were well.

These observations and those previously recorded by Fraser, *et al.* (14) are in accord with those of other investigators who have found the 17-ketosteroid excretion of chronically ill patients to be lower than normal (Chou and Wu (10) and later Chou and Wang (9) in hospital patients; Callow, Callow, Emmens, and Stroud (8) in chronically ill hospital patients; Moore, Miller and McLellan (21) in patients with benign hypertrophy of the prostate; Albright and Stewart (3) in a patient with steatorrhea and deficiency in all of the fat soluble vitamins; and Rhoads, Dobriner, Gordon, Fieser, and Lieberman (24) in patients with non-endocrine carcinoma).

In some metabolic and endocrine disorders where low values have been reported, the decrease is probably not due to the disorder per se but to the

accompanying secondary malnutrition. This may be the explanation of the low values reported by Fraser, *et al.* (14) in anorexia nervosa (see also, Fraser and Smith (15)), diabetes mellitus and hyperthyroidism; by Hamblen, Cuyler, and Baptist (18) in diabetes mellitus; by Miller and

TABLE 3. THE URINARY 17-KETOSTEROID EXCRETION OF 196 PATIENTS WITH CHRONIC DISEASE

Diagnosis	Number of Patients	Range in Age yr.	Average Age	Range in 17-Ketosteroid mg./24 hr.	Average Ketosteroid mg./24 hr.
			A. Males		
Malignancy (incl. prostate)	24	55–85	70	3.7–22.0	6.7
Sprue; Diarrhea	3	16–31	24	1.8– 2.9	2.3
Malnutrition; Anorexia	6	26–70	42	2.1– 7.6	4.5
Thyrotoxicosis	2	35–58	47	5.1– 5.3	5.2
Diabetes	6	15–71	45	1.8– 6.6	4.6
Hypertension	3	45–48	46	2.8–13.5	8.2
Rheumatic Disease	7	20–66	46	2.7–11.5	7.5
Other diseases	31	16–71	39	1.8–15.9	6.6
Total or Average	82		44		6.2
			B. Females		
Malignancy	5	15–70	48	1.5– 4.7	2.7
Sprue; Diarrhea	9	20–50	32	0.5– 8.3	3.3
Malnutrition; Anorexia	27	15–44	29	2.0–11.5	4.9
Thyrotoxicosis	17	18–60	44	1.3–17.3	5.1
Diabetes	9	28–68	55	2.4– 7.5	5.1
Hypertension	8	17–50	41	1.3– 5.3	2.7
Rheumatic Disease	12	16–48	31	1.2– 7.8	4.5
Other diseases	27	16–71	36	1.2– 9.5	4.2
Total or Average	114		37		4.4

Mason (20) in diabetes mellitus; and by Engstrom and Mason (12) in hyperthyroidism. Talbot and Butler (29) have summarized the situation with the statement that, "In chronic illness from any cause there is a tendency for the 17-ketosteroid output to be moderately lowered."

III. The 17-Ketosteroid Excretion of Previously Healthy Patients After Acute Trauma.

Whenever possible our studies have been carried out by making daily observations on a few individuals rather than scattered observations on a large group of patients. Figures 4 through 13 show the results of such in-

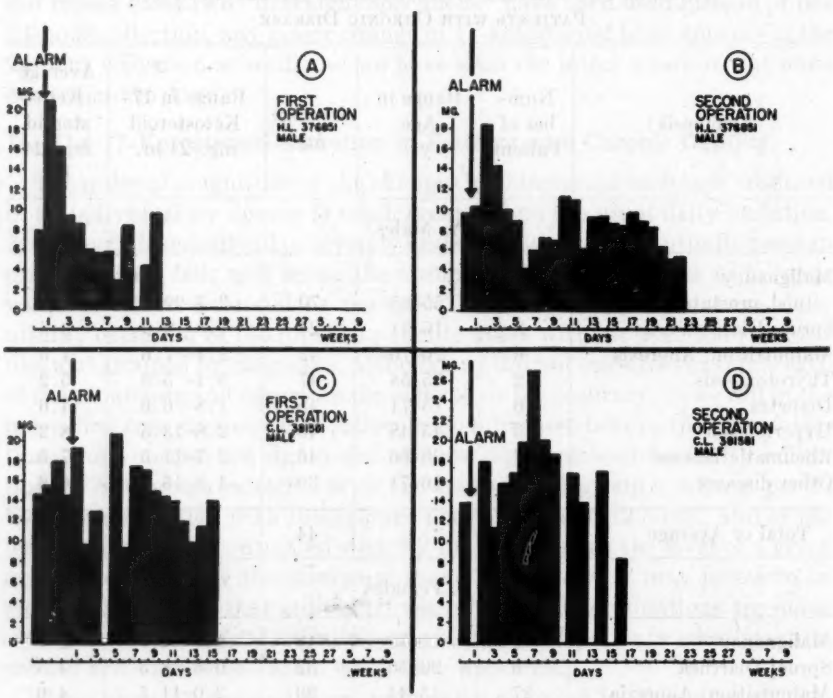

FIG. 4. The urinary 17-ketosteroid excretion of previously healthy men after operation.

Chart A illustrates the changes after the first stage, and Chart B the changes after the second stage of a bilateral lumbodorsal splanchnicotomy for essential hypertension in one individual. Charts C and D show similar data for another patient.

dividual case studies. These charts show that many kinds of trauma and stress affect the ketosteroid excretion according to the same, regular pattern.

Figures 4 through [illegible] illustrate the effect of surgical operations on previously healthy males. Elective operations were chosen because these allow time for establishing a preoperative baseline. The first three patients (Figures 4A and 4B, 4C and 4D, and 5A and 5B) had bilateral lumbodorsal

splanchnicotomy performed in two stages, the interval between the two operations varying from 12 to 14 days. Thus each of these patients furnishes us with two experiments. These men were 31, 40, and 26 years old, respectively, and except for essential hypertension were in good health. The fourth patient (Figure 5C) had a ruptured intervertebral disc removed.

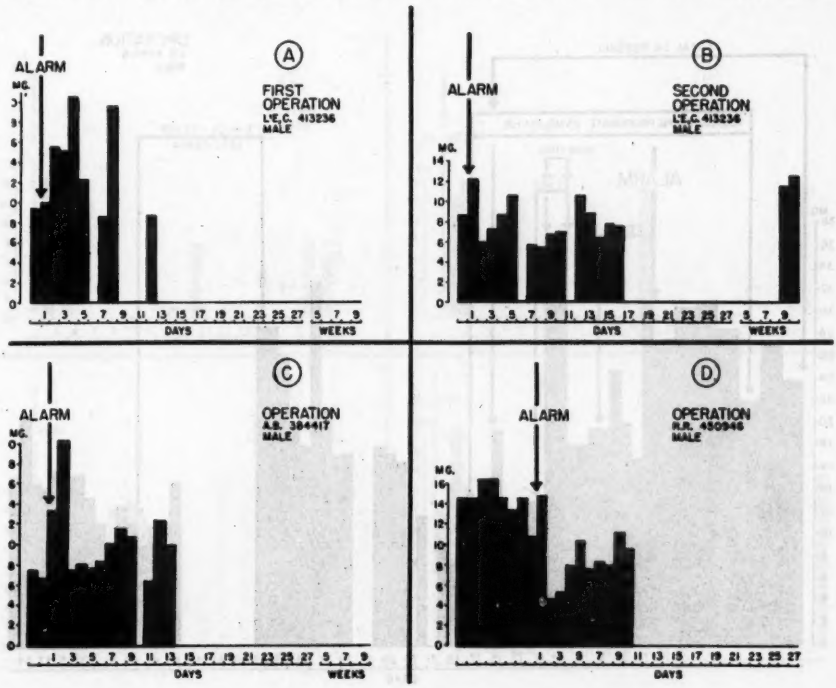

FIG. 5. The urinary 17-ketosteroid excretion of previously healthy men after operation.

Charts A and B represent the changes in the two stages of bilateral lumbodorsal splanchnicotomy of a patient. Chart C illustrates the changes after removal of a ruptured intervertebral disc; Chart D, the changes after a spinal fusion for spondylolisthesis.

He was a 41-year-old football coach, but had been unable to work for about a month because of back pain. The fifth patient (Fig. 5D), a 24-year-old man in good general health and able to chop wood, entered for a spinal fusion for spondylolisthesis. The sixth patient (Fig. 6), a 35-year-old man who was active as a gardener, also entered for a spinal fusion for spondylolisthesis.

The behavior of the 17-ketosteroid excretions was more or less alike in

all of these cases. Within 24 hours to 48 hours after the traumatizing event (operation) there was an increase in the 17-ketosteroid excretion to a point well above the normal limit. This peak usually occurred in the first specimen voided after operation (about 16 hours postoperative) but sometimes did not appear until later. After this the excretion fell rapidly and the low point usually occurred on the fourth or fifth day. Return of the excretion

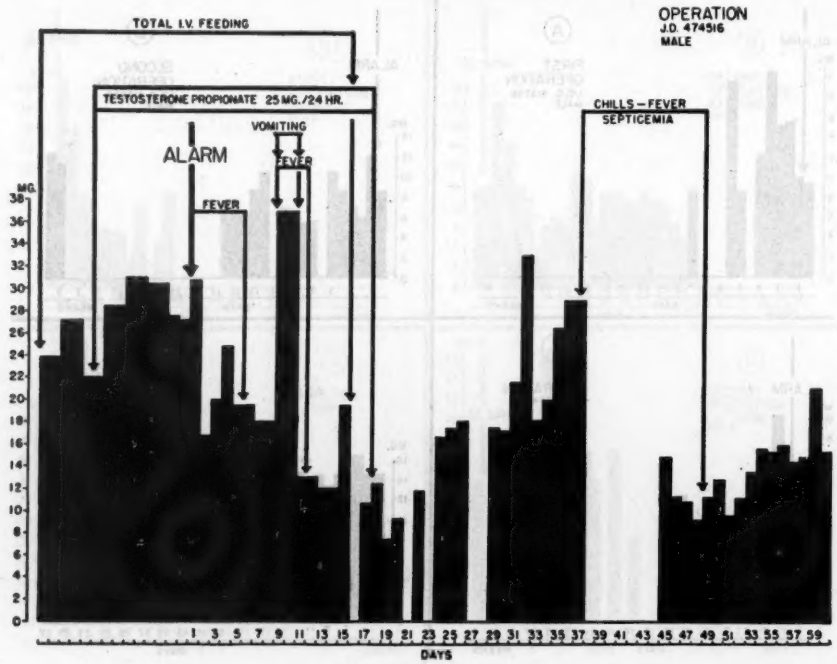

FIG. 6. The urinary 17-ketosteriod excretion after operation of a previously healthy man fed a constant intake and given testosterone propionate.

to a normal level occupied a more variable time, in these cases averaging ten days. Each individual behaved essentially the same way after the second operation as after the first; for instance the patient in figure 4C and 4D whose peak did not occur until the fifth day showed this same very unusual feature the second time. Similarly the patient in figure 4A and 4B showed a typical peak within 48 hours after both operations.

The studies on the fifth and sixth patients constitute strong evidence that the fall in 17-ketosteroids after an operation is not due to decreased food intake. Both were studied on the metabolic ward and both were fed a constant diet throughout entirely by vein. The fifth patient was kept in

bed for 8 days and the sixth for 14 days prior to operation. Their control values are, therefore, more adequate than those of the other four patients studied before and after major surgery. Both of these cases showed the expected pattern of 17-ketosteroid excretion after operation. Since testosterone propionate is partly excreted as a 17-ketosteroid, it should be noted that the sixth patient received 25 mg. of testosterone propionate intra-

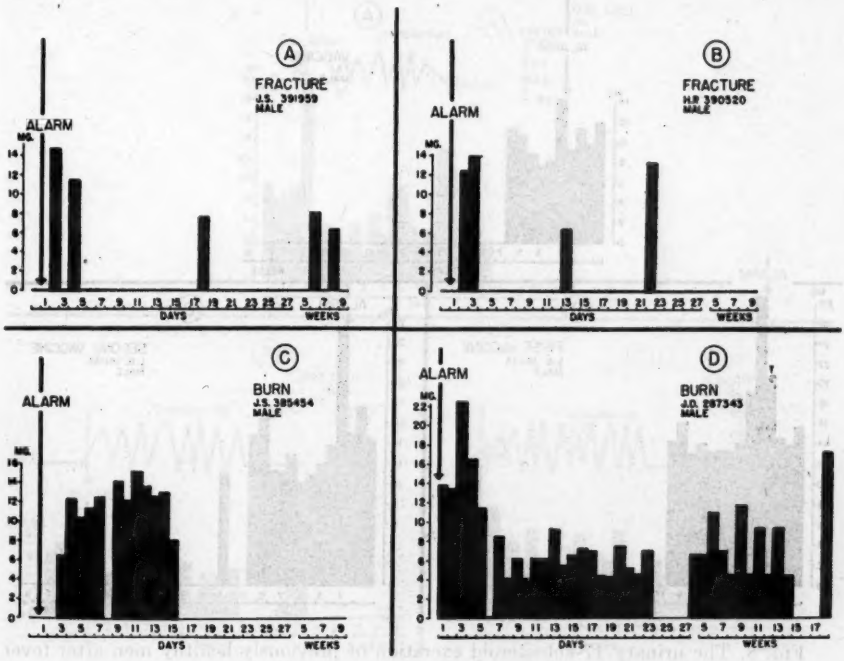

FIG. 7. The urinary 17-ketosteroid excretion of previously healthy men after fracture (A and B), and after burns (C and D).

muscularly each day for 9 days before and for 17 days after operation. Figure 6, in agreement with studies to follow, shows that other types of damaging events (vomiting and fever on days 9 to 11 from too rapid intravenous infusion, and chills and fever from septicemia on days 38 to 48) induce the same alterations in 17-ketosteroid excretion. The fifth and sixth patients have been referred to briefly elsewhere (Albright, Reifenstein and Forbes (2)).

Figures 7A and 7B show studies on normal males who sustained fractures. In this series there obviously could be no pre-injury control values.

Nevertheless, the curves appear to follow the same general pattern. Assays made before the third day fell in the high normal range and those made after the fifth day fell below normal or at least lower than after recovery. Return from these low levels appeared to be somewhat slower than after the sympathectomies.

The next two cases (Figures 7C and 7D) were brought to the hospital

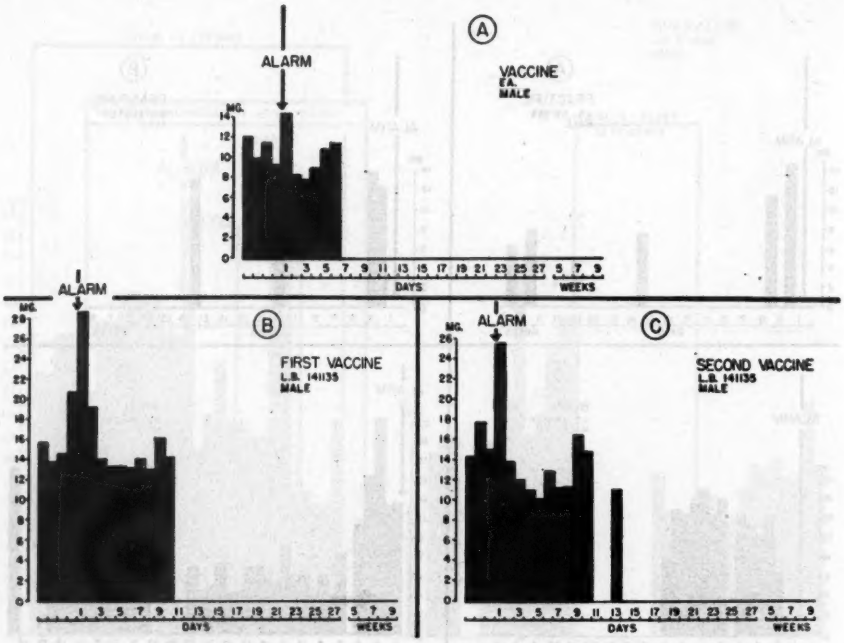

FIG. 8. The urinary 17-ketosteroid excretion of previously healthy men after fever induced by typhoid vaccine.

Charts B and C illustrate changes in two experiments in the same subject performed three months apart.

with burns. The first man was discharged in fifteen days in good condition. His 17-ketosteroid excretion rose from a level of 6 mg. per 24 hours on the third day after the burn to a level of 12 mg. per 24 hours within the first week. The other patient, however, was more severely burned. After an initial peak of 22 mg. per 24 hours on the third day, his excretion fell to between 4 and 7 mg. per 24 hours where it fluctuated for a month, during which time he was very ill. He was discharged after fifteen weeks, but his level was only 4 mg. per 24 hours on the day of discharge. One month later

a follow-up specimen yielded 16 mg. per 24 hours which is entirely normal for a male of this age. These observations agree with those of Cope, Nathanson, Rourke and Wilson (11) who studied these and other patients with burns at the time of the Cocoanut Grove Fire, and also with those of Stevenson, Schenker, and Browne (28) who studied a number of patients following various types of damage (burn, fracture, operation, infection and

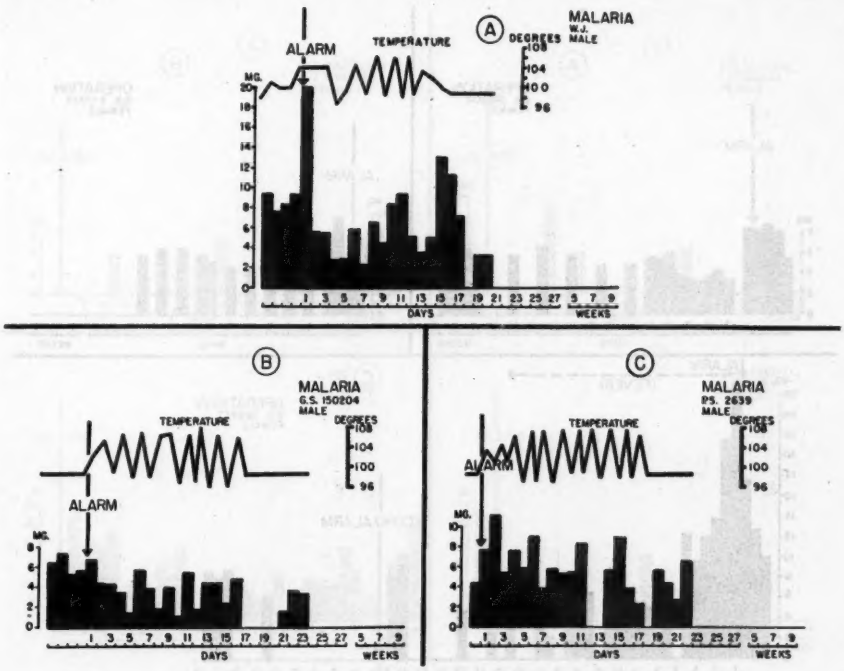

FIG. 9. The urinary 17-ketosteroid excretion of three men after fever due to malarial chills.

hemorrhage) at the Royal Victoria Hospital. The impression is gained from a study of patients with burns that the time required for the 17-ketosteroid excretion to return to normal is definitely related to the nature and severity of the injury and the amount of repair required.

Studies on various medical conditions confirm this impression. Figure 8A shows the effect of a single small injection of typhoid vaccine which produced fever for one day. The pattern of 17-ketosteroid excretion is much like that which follows a surgical injury, but a normal level is regained on the fifth day. Figures 8B and 8C show the results of giving much larger

doses of vaccine intravenously every day for three and for four days, respectively. The 17-ketosteroid excretion came back to normal on the ninth and tenth days. The last two experiments were performed on the same subject with an interval of three months between them. The subject was a healthy thirty-two year old man and the studies were carried out on the metabolic ward where his diet, activity and fluid intake were kept strictly

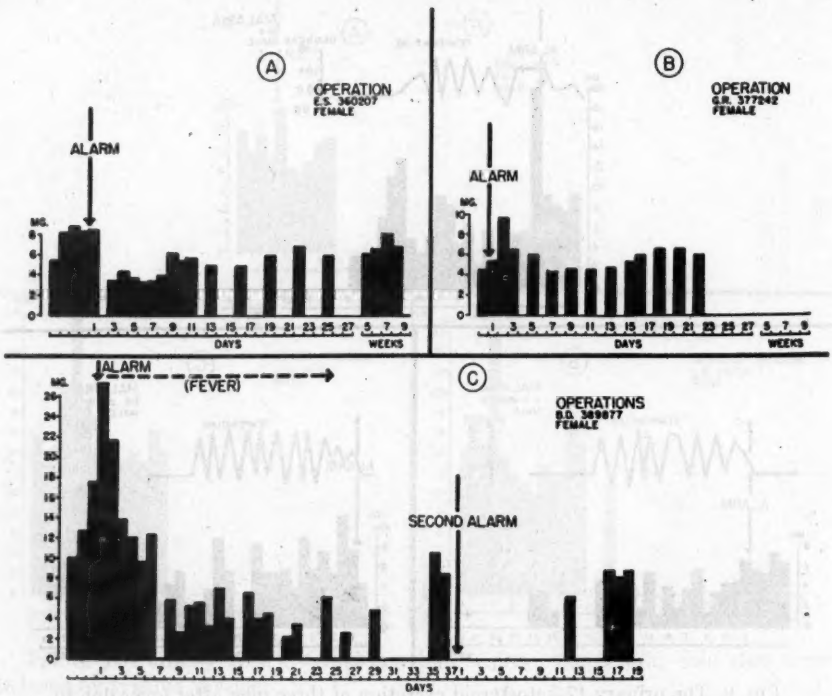

FIG. 10. The urinary 17-ketosteroid excretion after operation of two women who were normal (A and B) and of one woman who had obesity, hirsutism, and irregular menses (C).

constant. In a male laboratory worker who had influenza, the return to a normal level took over two weeks (see Figure 2C).

Since in spontaneous infections we seldom had an opportunity to establish the level of excretion before the infection began, we made some studies on patients who received malarial blood for cerebrospinal syphilis. Figure 9A shows the 17-ketosteroid excretion of a thirty-six year old man with tabes dorsalis who was in good general physical condition before treatment. The first malarial chill affected the 17-ketosteroid excretion in much the

same way as a surgical operation would have. Subsequent chills produced much less reaction and sometimes none. The pattern resembles that found by Howard and Bigham (19) to occur in the nitrogen loss of similar patients. This patient was severely prostrated by the malaria, and quinine was given on the thirteenth day of the disease because of debility. In two other patients (Figures 9B and 9C) there was a peak of 17-ketosteroid ex-

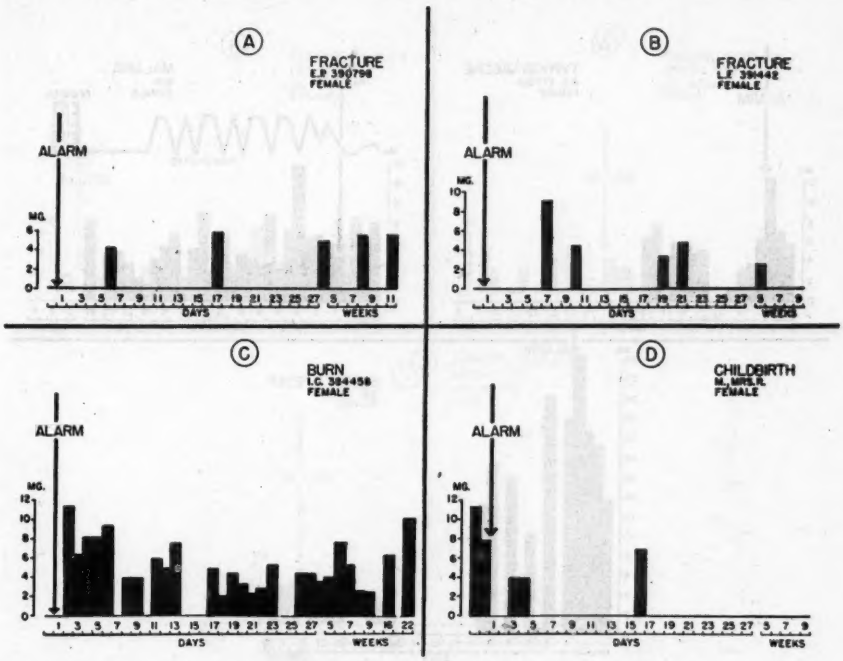

FIG. 11. The urinary 17-ketosteroid excretion of previously healthy women after fracture (A and B), burn (C), and childbirth (D).

cretion accompanying each malarial chill, but the peaks diminished progressively and the average level became lower and lower.

Figures 10A and 10B illustrate the effects of surgical operations on two healthy young women. The 17-ketosteroid curve was much the same as in males except that the peak which preceded the drop tended to be less pronounced. Figure 10C shows the excretion of another woman undergoing operation. This woman was obese and had hirsutism and irregular menses. She responded to operation with a peak of the same magnitude as the male cases in contrast to the women of Figure 10A and 10B. This may be characteristic of such women who usually have rather high 17-ketosteroid levels

normally (see also figure 12C). The point is of some interest since it supports the theory that we are measuring changes in the function of the adrenal glands and that changes in testicular function do not materially affect our results.

Figures 11A, and 11B show the excretion in cases of simple fractures in normal women; Figure 11C illustrates that of a severely burned woman.

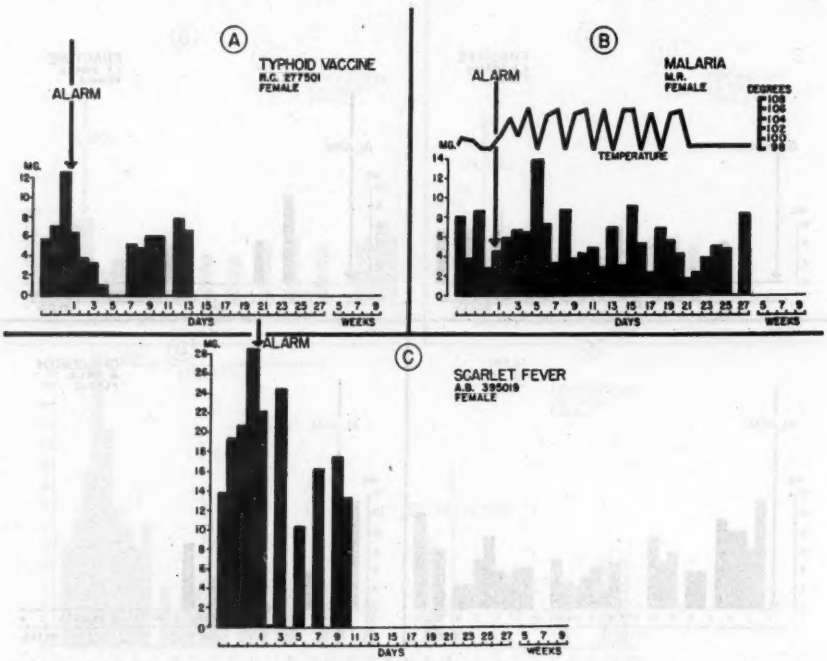

FIG. 12. The urinary 17-ketosteroid excretion of women with acute medical conditions: A—Fever due to typhoid vaccine, B—Malaria, and C—Scarlatinal wound infection in an obese, hirsute woman with irregular menses.

As in males, recovery following a severe burn was particularly prolonged. Figures 11D and 2B give the excretion of two women after normal childbirth. Here again there was a long recovery period, but in these cases other changes in endocrine status may have been playing a part.

The 17-ketosteroid excretion pattern of women with various medical conditions is illustrated by Figures 12A, 12B, and 12C. One of these, Figure 12 C, shows the reaction of an obese woman with hirsutism and irregular menses to a scarlatinal wound infection. Her normal excretion averaged 16.6 mg. per 24 hours which is not beyond the normal range, and

the infection caused a peak up to 28 mg. per 24 hours, which is decidedly high even for a male.

IV. The 17-Ketosteroid Excretion of Chronically Debilitated Patients After Acute Trauma.

We became interested in certain patients whose urinary ketosteroid excretion did not respond to injury with the pattern described above. The

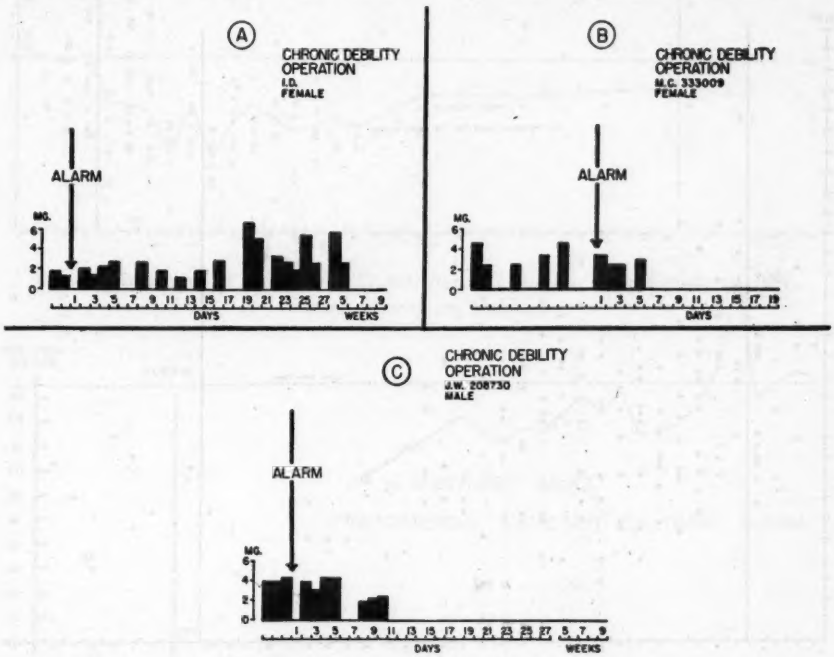

FIG. 13. The effect of acute trauma on the 17-ketosteroid excretion of three chronically debilitated patients: A. Woman with cerebro-spinal syphilis receiving malaria.* B. Woman with hypertension and congestive failure undergoing splanchnicotomy. C. Man with empyema undergoing surgical drainage.

* Incorrectly labelled operation.

first such patient whom we encountered (Fig. 13A) was a 43-year-old woman who received malarial blood for the treatment of cerebro-spinal syphilis. Her 17-ketosteroid excretion before treatment averaged 1.5 mg. per 24 hours. There was no peak and no significant fall after treatment although her temperature chart was similar to that of the other patients (see Fig. 12B). The second patient in this group, a 41-year-old colored woman with severe hypertension and cardiac failure, had an initial 17-ketosteroid level of 3.5 mg. per 24 hours before she underwent a sympathectomy.

There was neither a rise nor a significant fall (see Fig. 13B). A male patient who failed to respond is illustrated in Fig. 13C. He was a 30-year-old man admitted for drainage of an empyema with which he had been seriously ill for eight days before operation, who was in poor condition both before and after this procedure. The initial level of this patient was 4.0 mg. per 24 hours, which is a subnormal value. From these and other studies, we

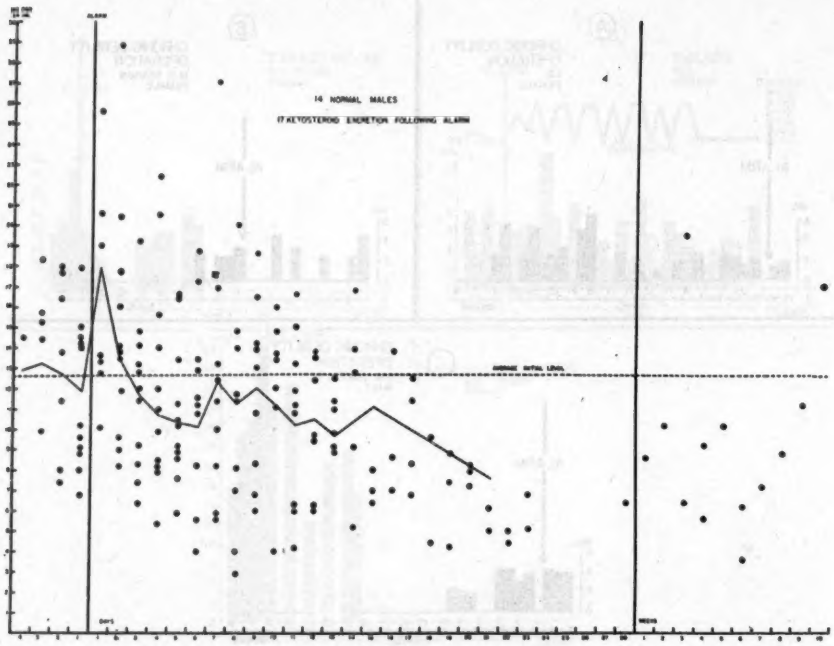

FIG. 14. Summary of the urinary 17-ketosteroid excretion of previously healthy men after acute trauma.

think that in those people who do not respond, the excretion level has already been lowered by chronic stress.

Figures 14 through 18 summarize the reactions of four different kinds of patients to injury. All of our cases both medical and surgical have been divided into four groups: (1) normal males; (2) normal females; (3) debilitated males; and (4) debilitated females. Patients of all sorts and ages have been included and the scatter is very great. The individual 17-ketosteroid values have been plotted against the number of days following the injury, and heavy lines have been drawn through the mean value of all of the patients on each day. These lines summarize our observations on the

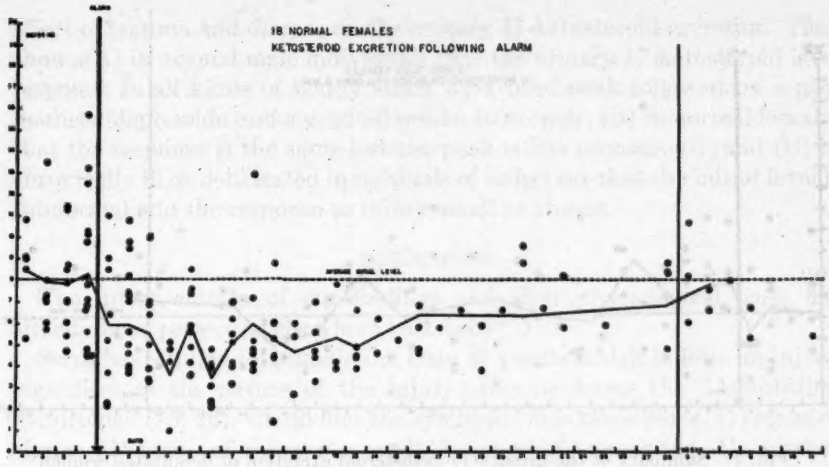

FIG. 15. Summary of the urinary 17-ketosteroid excretion of previously healthy women after acute trauma.

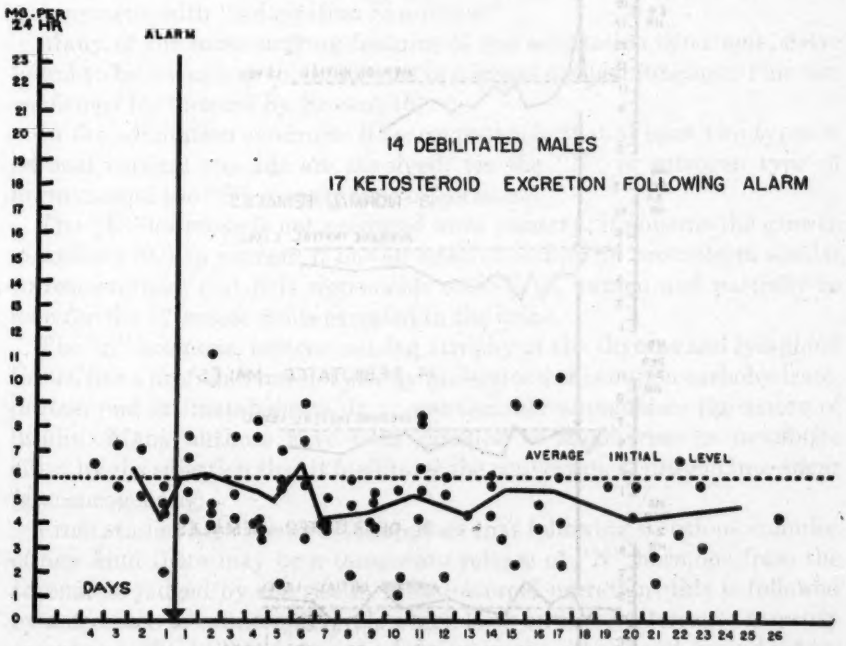

FIG. 16. Summary of the urinary 17-ketosteroid excretion of debilitated men after acute trauma.

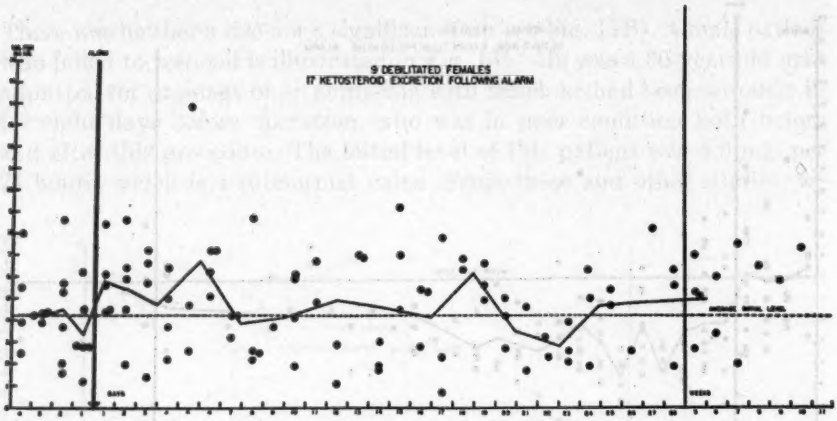

FIG. 17. Summary of the urinary 17-ketosteroid excretion of debilitated women after acute trauma.

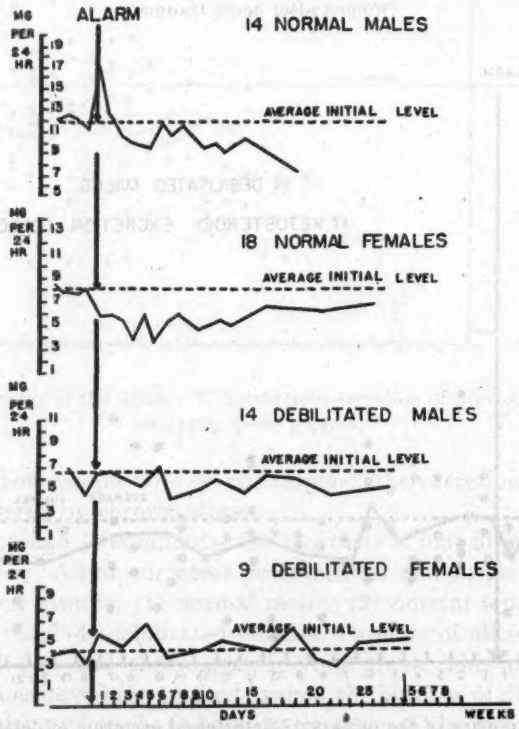

FIG. 18. The urinary 17-ketosteroid response of normal and debilitated men and women to acute trauma.

effect of trauma and disease on the urinary 17-ketosteroid excretion. They show: (A) in normal male individuals that the urinary 17-ketosteroid level responds to all kinds of bodily stress by a brief peak followed by a pronounced depression and a gradual return to normal; (B) in normal females that the response is the same but the peak is less pronounced; and (C) in chronically ill or debilitated individuals of either sex that the initial level is subnormal and the response to injury small or absent.

DISCUSSION

The interpretation of our findings and their physiological basis has already been presented elsewhere (Albright (1)).

Selye has studied in animals the train of events which follows an injury regardless of the nature of the injury; this he terms the "Adaptation Syndrome" (25, 26). He divides the syndrome into three parts: (1) stage of alarm; (2) stage of resistance, and (3) stage of exhaustion. He further divides the stage of alarm into two parts, shock and countershock. In clinical medicine it is not quite clear where one stage ends and another begins and there has been a tendency to use the term "Alarm Reaction" as synonymous with "Adaptation Syndrome."

Many of the most striking features of the adaptation syndrome, Selye found to be secondary to alterations in adrenal cortical function. This was confirmed for humans by Browne (6).

In the adaptation syndrome it seems probable that at least two types of adrenal cortical steroids are involved, viz the "N" or nitrogen type of hormone and the "S" or sugar type of hormone.

The "N" hormone is not produced until puberty; it governs the growth of axillary hair in women; it has an anabolic action on protoplasm similar to testosterone; and it is responsible entirely in women and partially in men for the 17-ketosteroids excreted in the urine.

The "S" hormone, besides causing atrophy of the thymus and lymphoid tissue, has a profound but not clearly understood effect upon carbohydrate, protein and fat metabolisms. It unquestionably antagonizes the action of insulin. Many authors have been satisfied to summarize its metabolic effect by the assertion that it facilitates the conversion of protein into sugar (gluconeogenesis).

From studies here presented it appears that following a noxious stimulus of any kind there may be a temporary release of "N" hormone from the adrenal as judged by the rise in 17-ketosteroid excretion; this is followed by a decrease to subnormal levels which lasts until convalescence is nearly completed. The "S" hormone production on the other hand, as judged by the excretion in the urine (biological assay (Venning and Browne (32)) and chemical assay of 11-oxysteroids (Talbot, Salzman, Wixom and Wolfe

(31)), rises with the "N" hormone but remains elevated until convalescence is practically completed (Venning and Browne (32)). The early phase in which both "N" and "S" hormone are released is probably analogous to the end of the countershock phase in animals when the adrenal cortices histologically show depletion of lipoid granules. The prolonged phase of low 17-ketosteroid excretion, coupled with continuous high "S" hormone excretion, probably corresponds to the phase of resistance. In those debilitated patients where an alarm is not followed by a rise in 17-ketosteroids it is probable that, due to a previous alarm, they are already in the stage of resistance or even approaching the stage or exhaustion.

The authors do not want to give the impression that the "N" and "S" hormones are the only adrenal hormones involved in the adaptation syndrome. Selye has indirect evidence that there is an associated increased excretion of the potassium-diuretic, sodium-conserving steroids similar to desoxycorticosterone. Smith and Smith (27) found a five to eightfold increase in the estrogen excretion of two postmenopausal and one surgically castrated woman following surgical operation.

SUMMARY

The urinary 17-ketosteroid excretion of sixty-six persons has been followed through some sort of medical or surgical injury. In normal individuals the urinary 17-ketosteroid excretion usually rises for a brief period (one to three days) and almost always falls for a longer period which lasts until convalescence is achieved when it gradually returns to normal. In chronically ill or debilitated individuals on the other hand, the initial level is subnormal and the response to injury usually small or absent. It is believed that these findings are further evidence that alterations in adrenal cortical function are an integral part of the adaptation syndrome of Selye and a link in our understanding of the part this syndrome plays in clinical medicine.

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THE DIAGNOSIS OF HYDATIDIFORM MOLE BY GONADOTROPIC HORMONE ASSAY USING THE SOUTH AFRICAN FROG, *XENOPUS LAEVIS**

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SOON after Zondek and Aschheim made their brilliant discovery of the mouse test for pregnancy (1), Shapiro and Zwarenstein (5), Hogben (3), and Bellerby (2) showed the clinical application of the South African Frog (*Xenopus laevis*) to the finding of gonadotropic hormones in the urine of pregnancy.

In its natural habitat, the female of this unique species of amphibia never lays eggs unless stimulated by the male. Shapiro and Zwarenstein (6) segregated the females from the males and found that ovulation and external egg deposition could be induced entirely at will by simply injecting concentrates of pregnancy urine into these ever-ready and ever-willing female *Xenopus*. And thus, the early foundation for the frog test for pregnancy was laid.

Since 1939, we in America have studied *Xenopus laevis* in detail and have performed thousands of pregnancy tests with the animal (7, 8, 9, 10, 11).[1] In our earlier papers we have reported our excellent results, and have registered our keen delight with *Xenopus* as a test animal for pregnancy. In 1944, we showed the African Frog to be invaluable as an indicator of pregnancy owing to its clear-cut end reaction (8). There is no such report as "doubtful." Eggs are either laid or they are not. The reaction can occur as early as four hours after injection but is usually demonstrated in between six and twelve hours. The test is inexpensive since the animal can be used over and over again. The end-result is simple; the eggs are macroscopic; they resemble caviar and can be seen easily even by the most inexperienced eye. In our hands, the frog test was shown to be ninety-nine per cent accurate in pregnancy diagnosis (11).

In recent years, the response of *Xenopus laevis* to excessive amounts of

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[1] For purposes of collecting in one source book all the available data on *Xenopus laevis*. the authors were fortunate in obtaining the assistance of the New York Biologic Research Foundation in 1944 in publishing their monograph entitled "The South African Frog (*Xenopus laevis*) in Pregnancy Diagnosis." This monograph contains 262 references on *Xenopus laevis* in which are included 30 references to work performed with *Xenopus* in America.

chorionic gonadotropin has been of use in diagnostic laboratory procedures other than pregnancy diagnosis. Thus, in 1944 Zwarenstein and Duncan (13), and Weisman and Coates (12) demonstrated the use of the animal in the diagnosis of hydatidiform mole; and in 1946 Meiring and Zwarenstein (4) described the diagnostic value of *Xenopus* in certain types of testicular tumors.

At this time, we are reporting in detail the use of *Xenopus laevis* in differentiating pregnancy from chorionic pregnancy tumors (hydatidiform mole). As is well known, these chorionic tumors are usually associated with tremendous outpourings of gonadotropic hormones in the urine. In most instances[2] the hormone found in the urine of a patient suffering with such a tumor is as much as a hundred or even a thousand times as great as is found in normal pregnancy. Occasionally, an investigator will find a low titre of hormone in a case of hydatidiform mole but this is the exception rather than the rule.

Xenopus has been of great help to us in facilitating the differential diagnosis between pregnancy and the tumors of pregnancy. Such a differential laboratory test is of great value when clinicians are presented with a patient, thought to be pregnant, but whose abdomen has grown out of proportion to the slow growth of a normal pregnancy, and who has begun to stain vaginally. In our pregnancy work with *Xenopus* (12), it required one cubic centimeter or more of urine (usually concentrates are required) injected under the skin to evoke the ordinary pregnancy reaction. Urine of pregnant women diluted 1:10 with water never contained enough hormone to cause a reaction. On the other hand, in our experience urine in a dilution of 1:10 from patients afflicted with hydatidiform tumors was consistently found to induce ovulation in *Xenopus*. In some cases a dilution of 1:100 induced strong reactions. This quantitative difference in hormone excretion led us to suggest the following quantitative test in preference to the much slower mouse test previously utilized in the differential diagnosis between early pregnancy and tumors of pregnancy.

TECHNIC

The comparatively simple method of assay follows. Two female *Xenopus* are placed in each of three small fish tanks which are one third full of water. The animals in the first tank, A, are each injected with one cubic centimeter of the undiluted suspected urine; those in tank B are injected

[2] From time to time, there will appear in the literature a report of a normal pregnancy which by hormone titre approaches the enormous amounts excreted in chorionic tumors. Clinicians must realize that any laboratory diagnosis is merely an adjunct to their clinical impressions, and that in the final analysis the results of hormone assays can only be used to confirm or deny the clinical diagnosis.

with one cubic centimeter of a 1:10 aqueous dilution of the urine; those in the third tank, C, are injected with one cubic centimeter of a 1:100 dilution. After four hours the tanks are inspected at regular intervals for the presence of ova.

If, after twelve hours, the animals in tank A (whole urine) react by extrusion and there is no reaction in tanks B and C, the diagnosis of simple pregnancy can be made with accuracy. However, if in addition to the reaction found in A, the animals in tank B (1:10 dilution) are found to be depositing eggs, the probability of a pregnancy tumor cannot be dismissed. If animals in all three tanks are found extruding ova, the clinical presence of a far-advanced chorionic tumor cannot be disputed. In this latter instance, the amount of hormone is in excess of 100,000 *Xenopus* units per litre of urine. No other condition in the differential diagnosis aside from chorionic tumor of pregnancy (either hydatidiform mole or chorionepithelioma) will evoke so strong a response.

RESULTS

Thus far, we have performed some thirty-one tests to differentiate pregnancy from hydatidiform mole. A tabulation of the results follows:

Reaction	*Diagnosis*
4 cases caused egg extrusion in all dilutions	—chorionic tumors
2 cases caused egg extrusion in 1:10 dilution	—chorionic tumors
25 cases evoked no response with undiluted urines	—no evidence of chorionic tumor
—	
Total 31	

Subsequently, the laboratory diagnoses were all substantiated clinically. The six cases which caused egg extrusion in any of the dilutions were found to be hydatidiform moles; of the twenty-five suspicious cases one was found at laparotomy to be a large ovarian cyst and the remaining twenty-four were definite pregnancies.

SUMMARY

A simple and rapid six to twelve hour method of assaying gonadotropic hormones in urine, using the South African Frog (*Xenopus laevis*), is presented.

Owing to the excessive amounts of this hormone found in the urine of patients suffering with hydatidiform moles (chorionic tumors of pregnancy) a dilution method of assay can be used successfully to differentiate these tumors from normal pregnancy.

Six cases out of thirty-one suspicious cases tested were successfully diagnosed preoperatively by this dilution method.

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MALE HYPOGONADISM TREATED BY SUBLINGUAL METHYLTESTOSTERONE

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THERE have been only a few reports on the clinical administration of methyltestosterone sublingually for treatment of male hypogonadism. One of the earliest was that of Spence, who reported a single case (9). Another concerned twelve male hypogonads ranging in age from 19 to 73 years (6). In all, satisfactory development of secondary sexual characteristics took place and was maintained on a lesser dosage schedule using sublingual methyltestosterone.* A subsequent report has confirmed the observations of others in regard to the effectiveness of relatively small doses of methyltestosterone sublingually (2).

Methyltestosterone sublingually may be administered in the form of Linguets or in a solution of propylene glycol. The former, however, is more practical than the solution, since solid material can be placed between the buccal mucosa and the tongue. In this locality, the Linguet usually remains unnoticed during the period of absorption, causing no inconvenience or restrictions beyond the admonition to refrain from "tonguing" the wafer and a temporary ban on eating during the time the Linguet remains in the mouth.

In previous reports the author has indicated the effectiveness of oral methyltestosterone in the treatment of male hypogonadism, (2, 3, 4). Since the advent of the Linguet form, a number of patients have been placed on this therapy, and the following case histories are typical of the response that can be expected. Sublingual administration of methyltestosterone ranged from 10 to 30 mg. daily in divided doses.

Case 1. C. E., age 36, married 8½ years, older brother of patient number 2. One uncle and two brothers are eunuchoids. The patient had a long history of cardiac disease and had been treated for cardiac dysfunction before coming to the clinic and being placed on testosterone therapy. It is of interest to note that cardiovascular imbalance has been reported to be frequently associated with hypogonadal types (8, 7).

Physical examination revealed bilateral cryptorchidism, extreme genital infantilism and chronic endocarditis. The patient's voice was markedly feminine. Previous therapy with testosterone had been irregular and insufficient.

Methyltestosterone, 30 mg. per day in the form of Linguets, was prescribed and this therapy continued for a period of five months. Within the first three weeks there was

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* The methyltestosterone tablets in the form of Linguets (Metandren) were supplied by the courtesy of Mr. Charles E. Munson of Ciba Pharmaceutical Co., Summit, N. J.

definite genital development (Fig. 1), and at the end of six weeks there was obvious deepening of the voice. Between the third and fourth weeks it was noted that both testes were beginning to descend. The patient reported marked improvement of vitality, and his endurance was considerably enhanced.

Although various reports have indicated that androgens produce favorable metabolic effects and that some of this may be on the basis of cardiotrophic action, it is a well known fact that androgenic therapy may produce augmentation of nervous and muscular

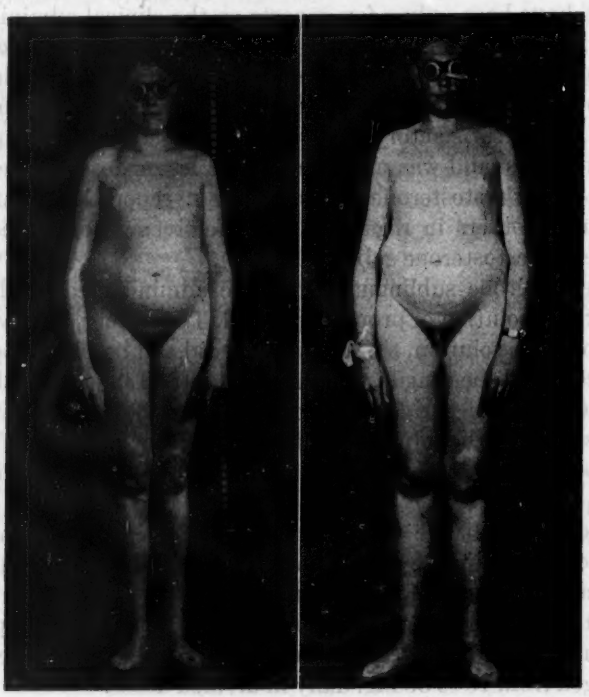

FIG. 1. C. E., aged 36, (*Case 1*). Improvement following two and a half months of therapy with Linguets.

activities which may be at variance with the moderation indicated by the condition of the heart and blood vessels, (5).

With the increased physical activity of this patient, his cardiac disorder became more marked; he developed a double murmur with partial decompensation and had to be confined to bed. It was deemed advisable to discontinue androgenic therapy because of the excessive stimulation in the physical sphere.

Case 2. J. E., age 28, married 4 years (see family history of older brother above). Physical examination revealed marked hypogenitalism, absence of facial hair, and scanty pubic and axillary hair. The patient talked in a very high-pitched voice. He complained of low energy and vitality. Previous therapy, which had been irregular and in-

sufficient, had consisted of intramuscular injections with testosterone propionate and chorionic gonadotropin, as well as pregnant mare serum. He had reacted well to testosterone injections with more frequent erections and a somewhat increased vitality, which, however, lasted only during the period of therapy. Other forms of treatment had produced no positive results. Linguets were started in a dosage of 15 mg. per day and continued for a month. At this time the dosage was decreased to 10 mg. per day, because the patient noted scrotal congestion and pain, whereas 1 Linguet (5 mg.) daily

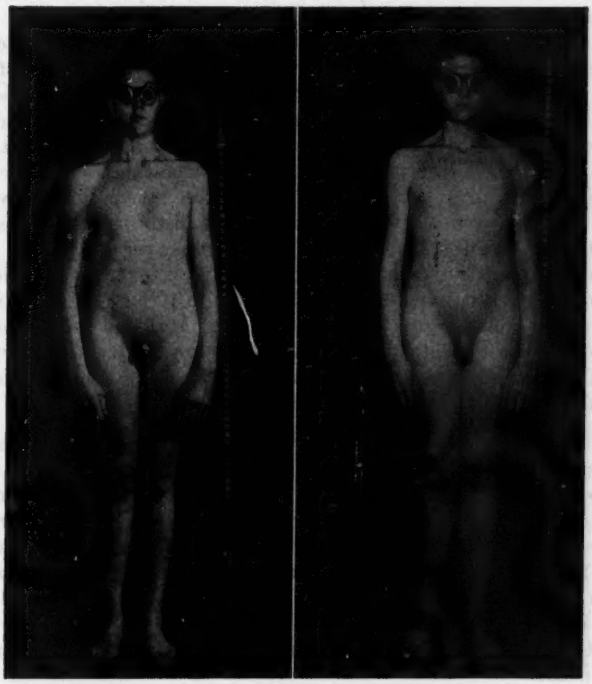

FIG. 2. J. E., age 28, (*Case 2*). Two and a half months of treatment with Linguets. J. E. is younger brother of C. E. (Case 1).

was not sufficient to cause any positive response. At the end of 17½ months the patient discontinued treatment of his own volition as he thought that it "increased his heart rate."

During the period the patient was being treated, there was noted a definite increase in the size of the genitalia (Fig. 2); there were an increased number of erections and obvious deepening of the voice. Pubic and axillary hair began to increase and there appeared a small amount of hair on the face and chest. At the end of 17½ months of therapy muscular development had increased to the extent that the patient required shirt size 15½, whereas before he required only 14½.

One year after discontinuing treatment, the patient showed some regression. During

treatment a small cystic mass, about the size of a cherry, appeared in the lower pole of the right testicle. This, however, gradually decreased and disappeared on cessation of therapy.

Case 3. J. G., age 21½, single. Physical examination revealed marked genital infantilism. This patient had been treated with pregnant mare serum for a period of about 2 months with no results whatsoever. He was then treated with testosterone propionate injections for a period of 1 month, totaling 250 mg. During this time, there was an increase in general muscular development, an increase of pubic hair, a deepening of the voice and gain in weight and vitality. Following this improvement, testosterone pellets totaling 300 mg. were implanted. One week later one pellet was expelled and later another pellet expelled. Two months later a second implantation of 300 mg. of testosterone pellets was carried out. Three months later he required injections of 25 mg. of testosterone propionate per week and these were continued for a period of about eight months, totaling 1223 mg. During the next nine months the patient received no therapy, but because of a recurrence of his symptoms oral methyltestosterone 20 mg. per day was prescribed and injections of 25 mg. of testosterone propionate were administered twice a week. There was a further increase in the size of the genitalia, accompanied by complaints of gynecomastia and three to four erections per day.

The patient was placed on Linguets, 15 mg. daily. It was found that this dosage caused too many erections and it was, therefore, decreased to 10 mg. per day. Linguets were used exclusively for economic reasons inasmuch as they are about half the cost of pellets.

On Linguet therapy the patient continued to improve and there was development of facial hair. The gynecomastia regressed. Excessive physical exercise was forbidden because of the detection of a faint heart murmur.

Case 4. L. M., age 30½ years, married 6 years. Physical examination revealed bilateral cryptorchidism, genital infantilism and absence of facial or body hair.

He had been treated with chorionic gonadotropin, thyroid, androsterone and testosterone, with only slight or moderate improvement. Testosterone therapy in 1936 had apparently caused some increase in the size of the genitalia and a moderate deepening of the voice. In October, 1944, testosterone pellets totaling 525 mg. were implanted. A reimplantation was done five months later with 300 mg. of testosterone and at four and five months, respectively, subsequent implantations of 300 mg. each. There was marked improvement in both the physical and sexual spheres following each implantation of testosterone pellets.

The patient was placed on Linguets, 15 mg. per day, and this dosage was maintained for one and one-half years. Remarkable improvement in the growth of the genitalia was noted (Fig. 3). There was rapid progress in regard to penile erections and emissions, as well as a rapid growth of body musculature and physical development. Facial hair and facial expression changed from a juvenile to a stern, masculine demeanor. When the patient took 4 Linguets per day, tachycardia was noted, and when the dosage was decreased to 2 Linguets per day, it proved insufficient to maintain progress. Cryptorchidism was not influenced by any therapy, though the presence of a small, soft body in the mid-portion of the right inguinal canal was noted occasionally. The patient changed to implantation treatment because of convenience only.

Case 5. E. P., age 43, single. This patient complained of extreme nervousness, inability to sleep, lack of concentration, occasional hot flashes, and frequent headaches. Physical examination revealed a rather obese individual, with evidence of surgical castration. History revealed that tuberculosis of both testes had necessitated their surgical removal

at the age of 20. Castration symptoms were brought under control with testosterone propionate injections in 25 mg. doses two to three times per week, and later with tablets of methyltestosterone 10 to 20 mg. per day. He was then placed on methyltestosterone Linguets 20 mg. per day; this was subsequently reduced to 10 mg. per day and therapy continued for two years with satisfactory results.

During this two year period a nephrectomy was performed for tuberculosis of the right kidney. Temporary withdrawal of the Linguet therapy resulted in a recurrence of

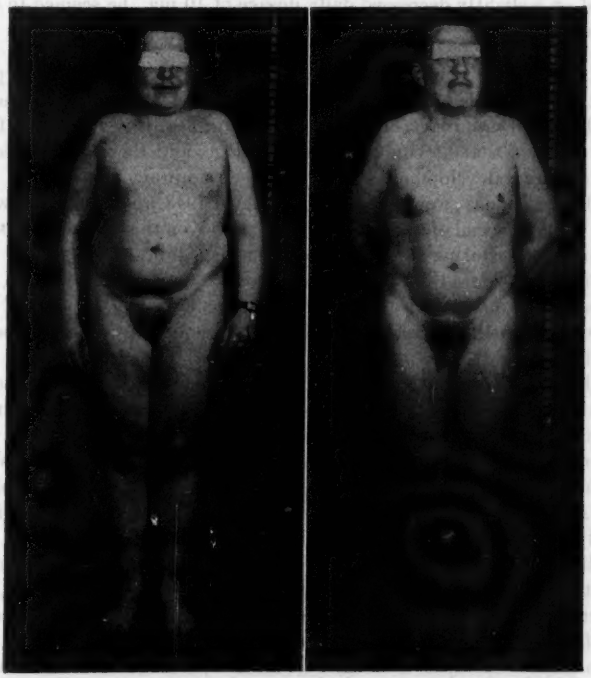

FIG. 3. L. M., age 30½, (*Case 4*). Originally treated with chorionic gonadotropin, thyroid, androsterone and testosterone with slight improvement. Pellet implantations produced marked improvement in physical and sexual spheres. Progress maintained and additional improvement on Linguets, 15 mg. per day.

symptoms, and the patient insisted on permission to resume the therapy a few days postoperatively.

There was general improvement in all of his symptoms on Linguet therapy; fatigue, cramps in the legs and arms disappeared. There was, however, no sexual stimulation as had been observed when taking methyltestosterone orally in doses of 10 mg. per day. Headache had recurred occasionally but there were no hot flashes; concentration was good. He found that the effect of 1 Linguet lasted from four to five hours.

Case 6. H. R., age 29, single. Physical examination revealed definite hypogenitalis,

absence of facial and body hair and a high-pitched voice. Previous therapy had consisted of a number of injections of testosterone propionate and repeated implantations of testosterone pellets. Initial priapism had been produced following the injection of testosterone propionate, 25 mg. three times a week. This was reported previously, (1).

Following implantation therapy, the patient experienced normal erections, normal libido, and was able to carry out normal sexual relations. In September 1944, the patient was given Linguets, 15 mg. per day, and continued on this therapy for a period of 7 months, during which time excellent results were reported, and although he could be maintained on a slightly decreased daily dosage of 10 mg., he seemed to do better on 15 mg. per day.

Case 7. M. Y., age 31, single. Physical examination revealed right cryptorchidism, genital infantilism, absence of body and facial hair, a moderately high-pitched voice and excessive girdle obesity and lack of energy and endurance. He expressed a feeling of insecurity and confided that he contemplated marriage, and therefore wished to achieve a fair degree of sexual development. There had been no previous therapy.

Linguets, 15 mg. per day, were prescribed in August 1943, and this dosage was increased to 20 mg. per day at the end of about ten days. Therapy on this schedule was maintained until December 1943. During the next three months a total of 750 mg. of testosterone propionate was administered in 25 mg. doses three to four times per week, because the patient was anxious to speed up the rate of development. This method of therapy did not seem to result in further increase in the size of the genitalia; it merely maintained the results already achieved.

While on Linguet therapy, there was a definite increase in the size of the left testicle the right testicle enlarged somewhat, but remained in the inguinal canal, and there was a gradual but definite increase in the size of the penis (Fig. 4), a deepening of the voice, an increase of pubic and axillary hair and some loss of adiposity. There was a definite increase in physical strength and endurance as well as in mental outlook and social adjustment. As the patient stated himself, he lost his shyness and feeling of inadequacy and acquired new interests in people and events.

Implantation of 300 mg. of testosterone was made in April 1944, in order to avoid the need of constant oral therapy, and the patient was married in June 1944, and able to carry on normal sexual relations. An additional implantation was made in September 1944, and a further implantation in February 1945. There was no further progress following implantation therapy, although the initial progress was well maintained. In July 1945, half of a pellet was expelled and in August another pellet was expelled. Pellets continued to be expelled, and because of this, Linguet therapy was resumed in December 1945. The original prescription of 15 mg. per day maintained the initial progress but produced no further increase in the development of the genitalia.

Case 8. E. B., age 21, was first seen on August 8, 1946, because of obesity and an arrest in genital development.

He was operated on at the age of 13 for bilateral cryptorchidism and an inguinal hernia. Prior to the operation, the patient was treated with Antuitrin "S" for two years. At the time of the operation, an atrophic left testicle was found in the inguinal canal and was placed in the scrotum; the right testicle was fairly well developed and was also brought down from the inguinal canal into the scrotum.

The patient began to gain weight steadily and in 1942, four years after the operation, he weighed 173 lbs.; there was no evidence of either testicle in the scrotum or the inguinal canal. The patient exhibited characteristic features of a eunuchoid, such as absence of

facial and body hair, scant pubic hair with female distribution, and lack of energy.

He was advised to begin glandular therapy, but he did not return to his physician until four years later (eight years following the operation) and was then advised to see an endocrinologist.

On initial examination, his height was 69½ inches, weight 206 lbs., pulse 80, blood pressure 112/60, heart and lungs normal. The body configuration was of eunuchoid proportions; there was an absence of facial and body hair, and the pubic hair line was

FIG. 4. M. Y., age 31, (*Case 7*). The above improvement obtained with Linguets, 20 mg. per day for four months. Patient subsequently treated with injections of testosterone propionate in an effort to speed up degree of development.

horizontal. The penis was abnormally small, scrotum shrunken and there was no evidence of testes either in the scrotum or in the inguinal canal. The voice was not high-pitched. There was complete lack of libido, erections and emissions.

The BMR was minus 22 and x-ray studies of the skull and sella turcica were within normal range. Roentgenological examination of the long bones showed failure of epiphyseal union of the radius and ulna.

The patient was placed on Linguets, 20 mg. per day, for six weeks, (this dosage was later reduced to 15 mg. per day), thyroid substance gr. 1 twice a day, a high protein, low fat and carbohydrate diet.

After four months of therapy, the patient showed a loss of 40 lbs., and an increase in energy and mental alertness, in growth of the external genitalia, and improvement in facial expression. (Fig. 5).

When last seen on October 22, 1946, the patient was very pleased with the progress

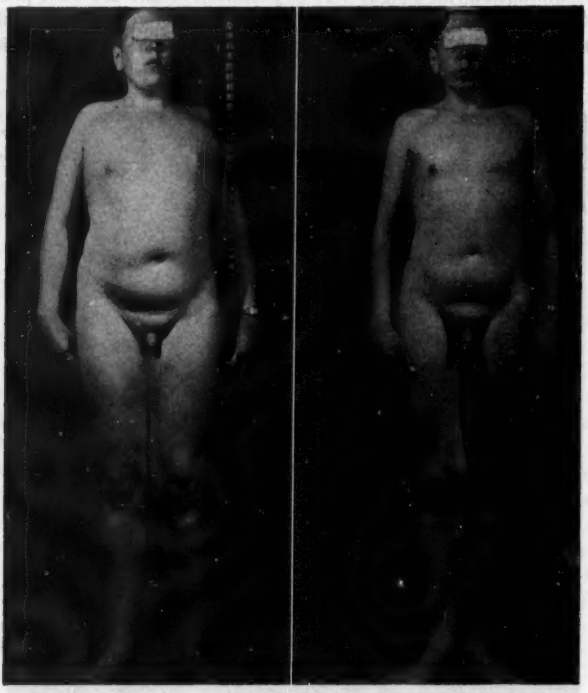

FIG. 5. E. B., age 21, (*Case 8*). Treated with Linguets and thyroid over a four-month period. Initially, 20 mg. of Linguets per day for six weeks and thyroid gr. 2 per day. Later, Linguets, 15 mg. per day. On this therapy the patient lost forty pounds in four months.

made and was eager to continue the therapy. The dosage of the Linguets was further decreased to 10 mg. per day, and the thyroid substance and diet continued.

The remaining four cases are briefly as follows:

Case 9. D. D., age 25, had been treated for hypogonadism, lack of energy, headaches and gynecomastia for the last five years with testosterone pellet implantations at periodic intervals. Because of his physical condition he was shy, reticent and did not mingle socially. He responded well to the testosterone therapy with an increase in energy, mental concentration, endurance and self assurance; however, he failed to develop facial hair to any extent. In order to achieve the latter, methyltestosterone Linguets 10 mg. daily were added to the implantation therapy. This resulted in a definite increase in facial hair. He was eventually placed on Linguet therapy exclusively, 15 mg. daily, with satisfactory maintenance.

Case 10. A. R., age 31, was treated with intensive chorionic gonadotropin therapy for genital and somatic infantilism, lack of energy, endurance and libido. A definite increase in the size of the genitalia occurred. There was great improvement in vitality, endurance and mental outlook. A moderate growth of hair appeared on the face and body. Because of the patient's desire to appear more masculine, he was placed on methyltestosterone Linguets 30 mg. per day; the original results achieved were maintained satisfactorily and the growth of facial and body hair was accelerated.

Case 11. L. Z., age 26, was treated with chorionic gonadotropin in large doses for cryptorchidism, hypogenitalism, obesity, debility, headaches and a high-pitched voice. He responded moderately well with a deepening of voice, increased energy, moderate increase in the growth of the genitalia and body hair. Since he still lacked endurance and was still subject to headaches, he was placed on methyltestosterone given sublingually 10 mg. daily, and responded favorably with an improvement in all the deficiencies mentioned above.

Case 12. W. D., age 22, a male pseudohermaphrodite, was treated intensively with testosterone in order to enhance the development of male secondary sex characteristics. The initial therapy consisted of injections with testosterone propionate and implantations with testosterone pellets. The therapy produced satisfactory results, but for the sake of convenience, he was placed on methyltestosterone Linguets, 20 to 30 mg. per day. This therapy maintained the original results achieved and caused further progress in muscular and genital development, deepening of the voice and an increase in energy and endurance.

SUMMARY

A group of 12 hypogonadal men (10 eunuchoids, 1 castrate, 1 male hermaphrodite) were treated with methyltestosterone tablets especially prepared for use by the sublingual route.

Each tablet contained 5 mg. of methyltestosterone; the daily requirement was from 2 to 6 tablets according to the needs of the patient.

The subjective and objective response of these patients was similar to that obtained with injections of testosterone propionate or implantations of testosterone pellets. This response can often be initiated or maintained on comparatively small dosage schedules.

In some of these patients the increased physical activity was at variance with the capabilities of the cardiovascular system, and the dosage was reduced accordingly; therapy was discontinued entirely in one case.

In all cases herein reported there was definite enlargement of the phallus and scrotum. A more male-like growth and distribution of body hair was observed. There was increased vigor, less tendency towards fatigue and a general personality change resulting in more masculine aggressiveness.

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Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor; D. A. McGINTY. *Collaborators:* A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

PITUITARY

CAPRIGLIONE, LUIZ AND JOSE SCHERMANN. Head trauma causing reactivation of thyrotoxicosis, appearance of diabetes mellitus, amenorrhea and melanoderma. *Arq. de clin. I:* 311 (1945).

The authors report the case of a white woman aged 36, with a history of the mother having diabetes mellitus and a sibling having epilepsy. There was a history of thyrotoxicosis following childbirth ten years previously which had subsided with conservative treatment. Six months before admission the patient was in a bus accident and lost consciousness. There was no report of a fracture. Soon afterward, polydipsia, polyuria and vulvar pruritus appeared. She began to lose weight, became nervous and tremors of the hands appeared. Pigmentary changes were observed. Clinical examination showed an enlarged, nodular thyroid; a small atrophic uterus and diffuse melanoderma. Her blood pressure was 110 systolic and 70 diastolic in a reclining position and 100 systolic and 60 diastolic in a sitting position. The basal metabolic rate was +22.5 and +34 per cent on two occasions. The blood sugar measured 250 mg.; the sodium, 350 mg.; and the potassium, 25 mg., per cent. The blood count was normal. Biopsy of the skin showed abundant melanin pigment in the histiocytes of the skin. Estrogenic substances in the urine measured 100 and 800 units in twenty-four hours. The authors believe the head trauma caused changes in the diencephalo-hypophysial region, resulting in exacerbation of thyrotoxicosis due to hypersecretion of thyrotropic hormones. The diabetes mellitus is considered a result of the effect of the hyperthyroidism on the pancreas already affected by the previous bout of thyrotoxicosis. The amenorrhea is said to be due to uterine atrophy induced by prolonged hyperthyroidism. The diffuse hyperpigmentation resulted from stimulation of secretion of intermedin, also a sequel of the head trauma.—*Abst. from Archiv. Neur. & Pschiat.*

HAY, E. C. The adrenotrophic, renotrophic and cardiotrophic activities of lyophilized anterior pituitary in thyroidectomized rats. *Am. J. Med. Sci. 212:* 535 (1946).

When adequate doses of lyophilized anterior pituitary suspension were administered for a sufficiently long time to thyroidectomized castrated rats, adrenotrophic, renotrophic and cardiotrophic responses were obtained which were statistically significant

even in the absence of the thyroid. However, adrenals, kidneys and heart appeared to be less sensitive to these trophic effects than in the presence of the thyroid.—*E.C.R., Jr.*

LUKENS, F. D. W. Pituitary-diabetes. *Am. J. Med. Sci. 212:* 229 (1946).

The author surveys the present state of affairs of pituitary-diabetes in terms of three aspects: 1) The identification of the hormones concerned with the metabolic activity of the pituitary; 2) the physiologic mechanism by which pituitary extracts, crude or refined, initiate the phenomena of diabetes; and 3) the relation of the pathogenesis of the permanent phase of diabetes with its obvious lesions of the islands of Langerhans to the preceding functional effects of the pituitary extract. Citation is made to 50 references. The author's summary follows. "According to studies done so far, no single purified pituitary hormone contains all the diabetogenic properties. Growth hormone is ketogenic and by the present limited criteria inhibits the utilization of carbohydrate. The adrenotrophic hormone mobilizes protein and carbohydrate and so is necessary for the phenomena of overproduction observed in diabetes. On theoretical grounds, maximum diabetogenic activity would result from a combination of these hormones, and this seems to be the case when crude extracts are employed. The mechanism of action of crude pituitary extracts is still poorly understood. However, this action includes evidence of the suppression of carbohydrate utilization and of overproduction under certain conditions. The effects of crude extract on glycogen storage, protein metabolism and fat metabolism compose a picture not incompatible with the gross observation that protein or carbohydrate food is necessary for the greatest diabetogenic effect. The suggestion has been made that the degree of glucose production and utilization needed by the fasting animal is so established or "fixed" by pituitary extract that carbohydrate or protein food cannot be metabolized in the normal manner and hence leads to hyperglycemia. If this hyperglycemia is sustained long enough, at least in the dog and cat, the islands of Langerhans undergo a sequence of structural changes resulting in irreversible damage and permanent diabetes."—*E.C.R., Jr.*

THYROID

ASTWOOD, E. B., AND W. P. VANDERLAAN. Treatment of hyperthyroidism with propylthiouracil. *Ann. Int. Med. 25:* 813–821 (1946).

Propylthiouracil was administered to 100 patients with thyrotoxicosis during the course of one year. The minimal daily dose of the drug necessary to restore metabolic equilibrium varied from 50 to 150 mg. Except for four instances of transient pruritis and one instance of urticaria, no toxic reactions were observed. Five patients who had developed febrile reactions on thiouracil and two patients who developed fever on thiobarbital were able to take propylthiouracil without untoward effect.—*J.M.*

BARMAN J. M. AND E. PORCILE. A simple method for assuring accuracy in determination of oxygen consumption by the Benedict-Roth apparatus. *J. Lab. & Clin. Med. 31*(11): 1254–1256 (1946).

In performing tests of basal metabolism in many patients with an irregular respiratory rhythm, it is difficult to draw the "oxygen line" accurately. Indeed, any of two or three lines may appear equally correct. Attempt is made to draw such lines through points of the curve corresponding to the end of expiration, at which time the lung contains both reserve (supplemental) and residual air. The volume of reserve air varies with

the depth of respiration. The depth of respiration, while commonly uniform in normal subjects, often varies considerably in diseased states, such as hyperthyroidism, and thus creates the difficulty mentioned above. To obviate this, the authors propose that at three or more times during the metabolic test the patient be instructed to exhale maximally, thus removing all supplemental air. Under such circumstances, they have found that the three or more points corresponding to the end of maximal expiration are in a straight line, and that oxygen consumption calculated with such a line as a basis corresponds closely to that obtained by gas analysis according to the Tissot-Haldane open circuit method.—*T.H.McG.*

BEIERWALTES, W. H., AND C. C. STURGIS. Complications following the administration of thiouracil. *Am. J. Med. Sci. 212:* 513 (1946).

From their experience in treating 80 cases of toxic goiter with thiouracil, the authors conclude that thiouracil is a valuable drug when employed preoperatively in patients with both nodular and hyperplastic toxic goiter. It is superior to iodine when used in this way because the basal metabolic rate may be brought to zero or lower, and also because it is equally effective in both types of toxic goiter. Iodine has not been highly satisfactory in the preoperative treatment of patients with toxic adenoma or in many cases with exophthalmic goiter especially when it has been previously administered, or possibly when the patients have ingested iodized salt over a long period of time. Only 1 patient in the group of 80 failed to show a basal metabolic rate below zero after thiouracil medication. The authors collected from the literature and by personal communication 10 fatalities occurring during or following thiouracil administration; of these 7 were definitely attributable to agranulocytosis. Analysis of these cases revealed a common pattern which gives clues to follow in the prevention of fatalities due to this complication. The authors recommend that the patient who is taking thiouracil be warned to discontinue the drug promptly following the appearance of fever with or without sore throat, skin rash or adenopathy. If a white blood cell count and estimation of the neutrophil percentage shows a significant depression of the cells, penicillin should be administered immediately until such time as the blood count becomes normal again. By such procedure the authors were able to avoid fatality in the three episodes of agranulocytosis that developed in their series. When the blood count becomes normal, thiouracil therapy may be resumed, but it is recognized that there is a definite risk of recurrence of the agranulocytic reaction. Other complications ultimately requiring thyroidectomy are febrile reactions, relative thiouracil resistance, nodular thyroid, large goiter, and failure of the patient to cooperate. The remaining complications (dermatitis, edema of the legs, jaundice, enlargement of the thyroid, production of accentuation of exophthalmos) occurring during thiouracil administration ordinarily do not require more than temporary cessation of the drug.—*E.C.R., Jr.*

DOBYNS, B. M. Exophthalmos and tissue changes in the guinea pig following administration of the thyroid stimulating hormone of the pituitary gland. *West. J. Surg. Obst. and Gyn. 54:* 411–427 (1946).

Exophthalmos was produced in guinea pigs by the injection of thyroid stimulating hormone, regardless of the presence or absence of the thyroid or testicles. A generalized alteration in fat metabolism occurred throughout the body, together with a connective tissue reaction. Fat depots were depleted and replaced by edema and connective tissue. Large quantities of lipoid appeared in the liver, kidneys, muscles, and in some epithelial

and reticulo-endothelial cells. It is suggested that many of the changes in the orbital tissue which produce exophthalmos are expressions of generalized tissue changes.—*J.M*

DOBYNS, B. M. AND S. F. HAINES. Changes in the prominence of the eyes in various thyroid states. *J. Lab. & Clin. Med. 31*(4): 483–484 (1946).

Thyroidectomy in patients with exophthalmic goiter usually was followed by increased exophthalmos. If hypothyroidism occurred postoperatively, the change was more marked. Treatment of both spontaneous and postoperative hypothyroidism decreased the prominence of the eyeballs. Thiouracil given to patients with exophthalmic goiter caused an increase in exophthalmos commensurate with the drop in basal metabolic rate. In the majority of all the studies, the changes were of such low magnitude as to be perceived only by careful exophthalmometry.—*T.H.McG.*

GORDON, A. S., P. C. KADOW, G. FINKELSTEIN, AND H. A. CHARIPPER. The thyroid and blood regeneration in the rat. *Am. J. Med. Sci. 212:* 384 (1946).

From studies in rats, the authors concluded: 1) thyroidectomy or thiouracil treatment inhibited the return of the red blood cells and hemoglobin to normal levels following bleeding. Studies of the leukocyte changes were inconclusive. Injections of thyroxin speeded regeneration of the red blood cells and hemoglobin to a greater degree in bled thyroidectomized than in bled unoperated rats. It is still an open question as to whether the thyroid directly regulates the erythropoietic process or whether this control is a secondary manifestation of the changes in general metabolism induced by the gland. 2) Castration in thyroidectomized animals did not further delay red blood cell regeneration, but caused a greater inhibition of hemoglobin synthesis. Testosterone was a more effective erythropoietic agent in the thyroidectomized-castrated than in the thyroidectomized rat. The combination of testosterone and thyroxin was little more effective than thyroxin alone. The authors believe that testosterone affects the erythropoietic process itself. Cobalt markedly stimulated red blood cell and hemoglobin production in bled thyroidectomized rats; the combination of cobalt and thyroxin was even more powerful. It is suggested that small amounts of this metal be administered along with the appropriate hormones in the treatment of clinical anemias accompanying endocrine deficiency. The hormones and cobalt, alone or in combination, all evoked marked reticulocytosis during the week following bleeding; but cobalt, and cobalt in combination with thyroxin, were the only treatments which maintained the reticulocytes at high levels throughout the experiment. Neither thyroidectomy nor administration of hormones or cobalt caused any significant change in the total or differential white cell counts of bled rats. The bone marrow of thyroidectomized rats displayed a slightly greater state of hypoplasia and a lower erythroid/myeloid cell ratio than that seen in unoperated animals for 3 to 4 weeks after the bleeding. Thyroxin tended to correct these changes; but cobalt, and especially the combination of cobalt and thyroxin, produce the greatest degree of marrow hyperplasia.—*E.C.R., Jr.*